

**ISOLATION, CHARACTERIZATION AND SCREENING OF
POTASSIUM-SOLUBILIZING BACTERIA FROM SOILS OF
VARANASI**

काशी हिन्दू
विश्वविद्यालय



**BANARAS HINDU
UNIVERSITY**

THESIS

Submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Soil Science and Agricultural Chemistry

Supervisor

Prof. B. R. Maurya

Submitted by

Vijay Singh Meena

**Department of Soil Science and Agricultural Chemistry
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*Isolation, Characterization and Screening of Potassium-
Solubilizing Bacteria from Soils of Varanasi*

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy
in
Soil Science and Agricultural Chemistry

2016

by

Vijay Singh Meena

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PREFACE OF THE THESIS

This thesis is written as the final draft for doctoral degree in Soil Science and Agricultural Chemistry with the primary aim to find out **Isolation, Characterization and Screening of Potassium-Solubilizing Bacteria from Soils of Varanasi**. I hope the results will be of interest to soil scientists, agricultural microbiologist and environmentalists for boosting research on potassium solubilization and maintenance of soil, crop and environmental quality and soil as well as human health.

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Chapter V summarizes the research work and the conclusions drawn from the present investigation.

The section on Bibliography embodies references consulted during the entire course of investigation.

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LIST OF ABBREVIATIONS

%	Per cent
@	At the rate of
<	Less than
>	Greater than
°C	Degree centigrade
BOD	Biological oxygen demand
CD	Critical difference
cfu	Colony forming unit
cm	Centimetre
DAI	Days after incubation
DAS	Days after sowing
DMRT	Duncan's Multiple Range Test
dSm ⁻¹	Decisiemen per meter
e.g.	Example
EM flask	Erlenmeyer flask
<i>et al.</i>	<i>et al.</i> (co-authors)
FYM	Farm Yard Manure
H ₂ S	Hydrogen sulphide
HCN	hydrogen cyanide
i.e.	<i>Id al.</i> (that is)
IAA	Indole-acetic acid
KSB	Potassium solubilizing bacteria
KSM	Potassium solubilizing microorganisms
KSR	Potassium solubilizing rhizobacteria
MUB	Modified Universal Buffer
NPK	Nitrogen, Phosphorous, potassium
OD	Optical density
PCA	Principal component analysis
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
SD	Standard deviation
SE	Standard error
SEm	Standard error of mean
<i>Viz.</i>	Namely
WB	Waste biotite
WM	Waste muscovite
ZS	Zone of solubilization

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INTRODUCTION

As the world population continues to increase at an alarming rate, the demands placed upon agriculture to supply future food will be one of the major challenges facing the human population. In order to meet this challenge, a great deal of effort focusing on the soil biological system and the agro-ecosystem as a whole is needed to enable better understanding of the complex processes and efficient interactions between soil-plant-microorganisms governing the sustainability of agricultural soils. There are several minerals which contain third essential plant nutrient element “potassium” in soil. Potassium plays a key role in the growth, metabolism and development of plants. Without an adequate supply of potassium, the plants will have poorly developed roots, grow slowly, produce small seeds and have lower yields as well as susceptible to diseases, insect and insects (White and Karley, 2010; Armengaud *et al.*, 2010; Troufflard *et al.*, 2010).

Due to luxury consumption of K required in large amounts by plants and many agricultural soils lack sufficient phyto-available K for maximal crop production (Rengel and Damon, 2008). It is generally supplied as K-fertilizers in both intensive and extensive agricultural systems (Phua *et al.*, 2012; Yadegari *et al.*, 2012; Zhang *et al.*, 2013). According to Hasan (2002), potassium fertility status of Indian agricultural soils is categorized as low (21%), medium (51%) and high (28%). Thus, ~ 72% of India's agricultural area, representing 266 districts which need immediate K fertilization for healthy crop production because such imbalances of K are widespread in agricultural soils and particularly show in sandy and lateritic soils due to leaching (Mengel and Kirkby, 2001; Rengel and Damon, 2008).

The microorganisms play a central role in natural K and P cycles and potassium and phosphorus solubilizing bacteria (PSB) in soil as well as in the plant rhizosphere (Diep and Hieu, 2013). Balanced K fertilization and avoidance of K mining, (K applied by fertilizers less than that K removed by crop harvest) will prevent farmers from falling into the poverty trap and will help them leave the vicious circle of declining soil fertility as the K content of the crop residue has been always much higher in comparison to N and P (Krauss, 2003).

Soil-plant-microbe interaction has got much importance in recent decades. Many types of microorganisms are known to inhabit soil, especially rhizosphere and play important role in plant growth and development. There are abundant microorganisms thriving in soil, especially in the rhizosphere. It is well known that a considerable number of microorganisms (bacterial and fungal) species possess a functional relationship and constitute a holistic system with plants. They are able to easily multiply in a rhizosphere to promote plant growth and yield (Vessey, 2003). Farmers apply chemical fertilizers in unmanaged and an unbalance way for crop production because they are not aware about the quantity of fertilizer necessary for plant that varies from crop to crop.

There is a very big gap between researchers and farmers. Most of the farmers only use urea as nitrogen, some Di-ammonium phosphate (DAP) as phosphorous but only few of them use K-fertilizer as muriate of potash (MOP) for crop production. Therefore, available forms of potassium decrease in soil due to luxury consumption by the crop. However, crop residue has more potassium content than other elements. Nowadays farmers are not adding crop residue in soil that is one considerable reason for the depletion of potassium in soil systems, which ultimately shows the poor crop performance. To mitigate this and to maintain the fertility status of soil, the balanced use of chemical fertilizers is needed, though it is found to be a costly affair and also environmentally undesirable (Mohammadi and Sohrabi, 2012; Zhang and Kong, 2014; Meena *et al.*, 2015).

K-solubilizing bacteria are able to release potassium from insoluble minerals (Zarjani *et al.*, 2013; Maurya *et al.*, 2014; Zhang and Kong, 2014). In addition, researchers have discovered that K-solubilizing bacteria (KSB) can provide beneficial effects on plant growth through suppressing pathogens and improving soil nutrients and structure. Certain bacteria, weather silicate minerals to release potassium, silicon and aluminum and secrete bio-active materials to enhance plant growth. These bacteria are widely used in biological K-fertilizers and biological leaching (Lian *et al.*, 2008; Meena *et al.*, 2013; Meena *et al.*, 2015).

The considerable populations of KSMs are present in rhizospheric soils which promote the plant growth. It is generally accepted that the major mechanism of the mineral K-solubilization is the action of organic acids synthesized by rhizospheric microorganisms. Production of organic acids result in acidification of the microbial cell and its surrounding environment which promote solubilization of mineral K. Silicate bacteria were found to desolve potassium, silicon and aluminum from insoluble minerals (Aleksandrov *et al.*, 1967). Silicate bacteria exert beneficial effects upon plant growth and yield. The KSMs can promote K-solubilization from silicate mineral is very important to enhance the fertility status of soils. Rhizospheric microorganisms contribute directly and indirectly to the physical, chemical and biological parameters of soil through their metabolic activities.

The rhizospheric bacteria help in soil processes such as exudation of soluble compounds, storage and release of nutrients, mobilization and mineralization of nutrients, soil organic matter decomposition and solubilization of K (Zeng *et al.*, 2012; Meena *et al.*, 2014b), phosphate solubilization, nitrogen fixation, nitrification, denitrification and sulfur reduction (Khan *et al.*, 2009; Diep and Hieu, 2013). Those bacteria possessing potassium solubilizing ability are called potassium solubilizing bacteria (KSB) and they can convert the insoluble or mineral structural potassium compounds into soluble forms in soil and make them available to the plants (Zeng *et al.*, 2012).

Imbalanced or overdose of chemical fertilizers have the negative environmental impacts and also increase the costs of crop production, therefore, there is an urgent need to employ ecofriendly and cost effective agro-technologies to increase crop production. Therefore, the utilization of KSMs is considered to be a sound strategy in improving the productivity of agricultural soils. This novel technique is also claimed to show the ability to restore the productivity of degraded, marginally productive and unproductive agricultural soils (Maurya *et al.*, 2014; Meena *et al.*, 2015). However, utilization of KSMs is found to be limited because lack of knowledge among the farmers and practitioners (World Bank, 2008).

The potassium content of Indian soils has traditionally been considered as adequate, but in the recent years, the importance of K and the need for its continued optimal availability for the better crop production has been observed as deficient due to the hidden hunger and luxury consumptions of K (Leaungvutiviroj *et al.*, 2010; Zhang and Kong, 2014). Among the major plant nutrients, potassium is one of the most abundant elements in soil. It is one of the seven most common elements in the earth's crust and on an average the surface layer (lithosphere) contains ~ 2.6% potassium. The present conceptual understanding of soil potassium availability is the existence of four distinct K pools differing in accessibility to plant roots with the reversible transfer of K between the pools. The K content of Indian soils varies from less than 0.5 to 3.0%. The average total K content of these soils is ~ 1.52% (Mengel and Kirkby, 1987).

Soil solution K plays a vital role in providing the pathway for K uptake from the soil by plant roots (Oborn *et al.*, 2005). According to McLean and Watson (1985) this pool is very low in K content, representing only 0.1-0.2% of the total soil K (~ 5% of total crop demand). It is immediately available and replenished by both the exchangeable K (EK, readily plant available K) and the slow or non-exchangeable K (SEK, slowly plant available K) pools. These two pools, EK and SEK make up about 1-2% and 1-10% of the total K, respectively and are the main contributors to K uptake by plants. The exchangeable fraction, i.e. the K held on negatively charged sites of clay minerals and

soil organic matter is in rapid equilibrium with soil solution K and is considered to be readily available to plants. These rhizospheric phenomena between soil-microorganism-plant as affected by a potassium solubilizing microorganism (KSMs) is presented on the Fig. 1.1. Remaining pool or major pool of K which holds the bulk ~ 90-98% of the total soil K, is held in the structure of the primary K bearing mineral soils, K occurs in the form of silicate minerals viz., muscovite, orthoclase, biotite, feldspar, illite, mica (ruby mica, mica powder, mica scrap, mica stone, mica flakes), vermiculite, smectite. The total pool of soil K is extremely complex and this can be solubilized by KSMs through the production of acids and it will be available to plants (Basak and Biswas, 2012).

A wide range of rhizospheric microorganisms is reported as K-solubilizers include *Bacillus mucilaginosus* (Zhao *et al.*, 2008; Sugumaran and Janarthanam, 2007; Zarjani *et al.*, 2013), *B. edaphicus* and (Sheng, 2005), *Burkholderia*, *Acidithiobacillus ferrooxidans*, *B. mucilaginosus*, *B. edaphicus* (Sheng and He, 2006) *Arthrobacter* spp. (Zarjani *et al.*, 2013), *Enterobacter hormaechei* (Prajapati *et al.*, 2013); *Paenibacillus mucilaginosus* (Liu *et al.*, 2012), *Pseudomonas frequentans*, *Clasdosporium* (Argelis *et al.*, 1993); *Paenibacillus glucanolyticus* (Sangeeth *et al.*, 2012). These microbial strains have the ability to solubilize of K from K-bearing minerals, but only a few bacteria, such as *B. mucilaginosus* and *B. edaphicus* have high activity in mobilizing and solubilizing of K from minerals (Lian *et al.*, 2002; Li *et al.*, 2006; Zhao *et al.*, 2008; Rajawat *et al.*, 2012). These bacteria have wide applications in mining, metallurgy, microbial fertilizer, and feed (Liu *et al.*, 2012; Zhang and Kong, 2014).

Maize (*Zea mays* L.) is one of the most important cereal crops in the world that maintain the world agricultural economy. It belongs to *Poaceae* family and is the only species of the genus *Zea* (2n=20). The outstanding features of maize have been well documented all over the world. This crop is being considered as a “*Queen of Cereals*”. Maize is being consumed in many ways; such as human food, fodder for animals and in industry for production of milk and oil. It ranks below wheat and sorghum, but considerably above rice as far as the nutritive value is concerned.

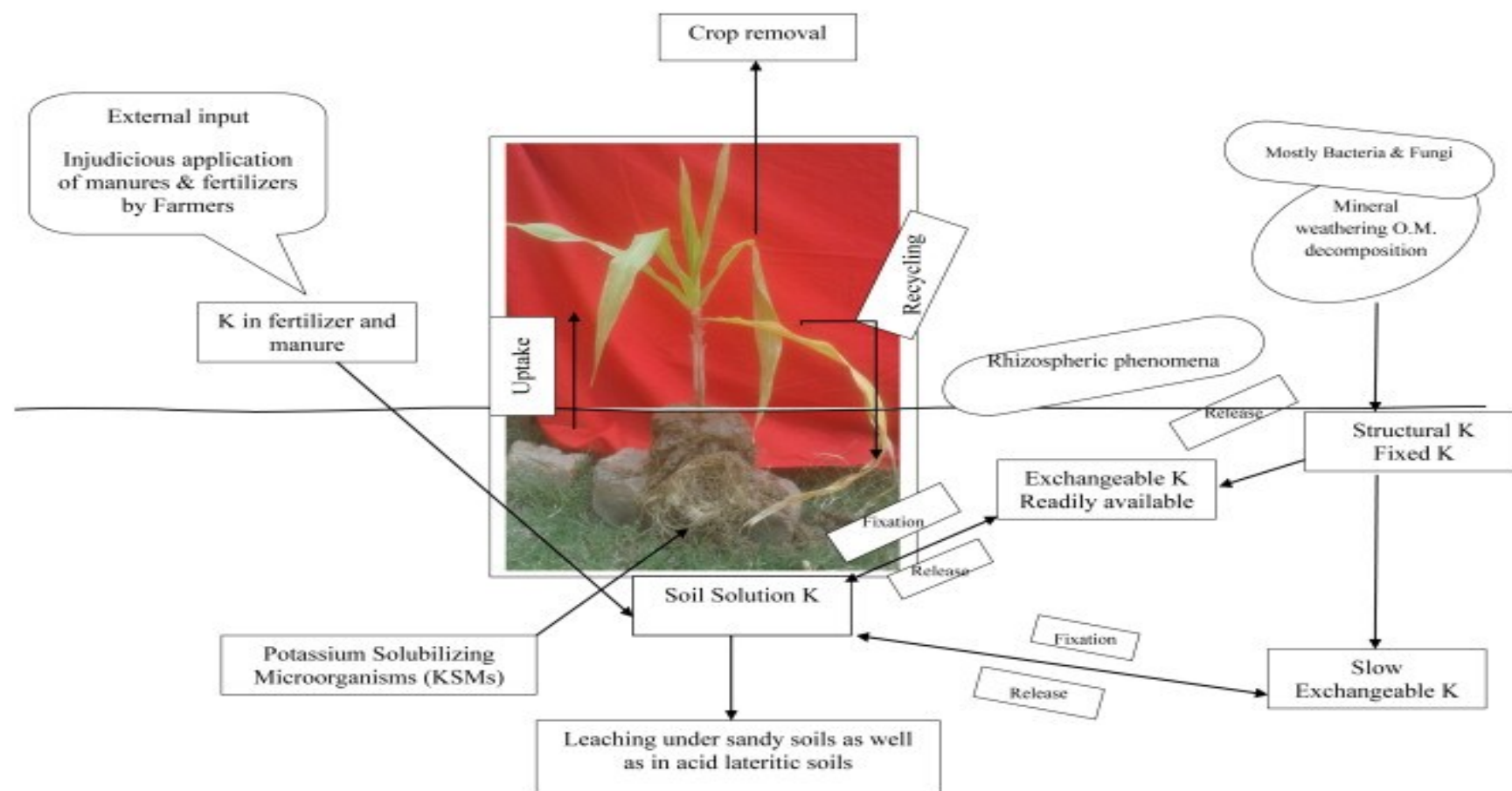


Fig.1.1: Rhizospheric phenomena between soil-plant-microorganism as affected by potassium solubilizing microorganism (KSMs)

Maize grains contain 10% protein, 4% oil, 70% carbohydrate, 2.3% crude fiber, 10.4% albuminoids and 1.4% ash. Maize grains have a significant amount of vitamin A, nicotinic acid, riboflavin and vitamin E. Maize is widely cultivated throughout the world. The United States produces ~ 40% of the world's harvest; other top producing countries included the China, Brazil, Mexico, India, France and Argentina. In India, maize occupies third position both in area and production after rice and wheat. It is grown over an area of ~ 8.26 M ha with total production of ~ 16.72 MT/year (Anonymous, 2014). In India major producing states, Andhra Pradesh tops the list with the contribution of ~ 17% to the total Indian maize production. Other producers are Rajasthan (14%), Madhya Pradesh (12%), Bihar (10%), Uttar Pradesh (9%), Karnataka (8%) and Gujarat (6%). Maize requires 120 kg N, 60 kg phosphorus and potassium 60 kg potassium/ha as chemical input for required maize production. This amounts to be high input crop and farmers have to invest money heavily. Annually, more than 143.88 MT of chemical fertilizer is used worldwide to increase the yield of crop plants. Despite their efficiency in promoting crop yield, they have proved to be hazardous for soil health as well as for the well being of human and animal populations.

The current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizer, forcing the search for alternative ways to improve crop yield in sustainable agriculture (Abou-el-Seoud and Abdel-Megeed 2012; Meena *et al.*, 2015). The biological fertilizer is, therefore, preferred over chemical fertilizer, as they are not only ecofriendly and economical in approach, but are also involved in improving the soil quality and maintenance of natural soil flora. The use of microbial inoculants as biofertilizers and/or antagonists of phyto-pathogens are a tool of sustainable agriculture and provide a promising alternative to chemical fertilizers and pesticides (Asghar *et al.*, 2002; Zhang and Kong, 2014).

Soil-plant-microbes interaction has been widely accepted as an alternative to chemical fertilizers for enhancing agricultural productivity along with its sustainability, and is used as a low-input, cost effective technology for enhancing soil fertility in countries like India, where the soil has become exhausted by the need to

produce more food for increasing populations (Hazell and Wood, 2008; Nadeem *et al.*, 2014).

The aim of the study is to explore our own soils for identifying potassium solubilizing bacteria suitable for soils of Varanasi. This work will help in the development of bacterial inoculants to use as potassium solubilizing bacteria that will be feasible and commercially viable. This work will emphasize to find out the suitable K-solubilizing bacteria to enhance the supply of potassium by transforming insoluble forms of potassium into soluble forms. The work entitled **“Isolation, Characterization and Screening of Potassium-Solubilizing Bacteria from Soils of Varanasi”** was carried out with the following objectives.

1. Isolation of potassium solubilizing bacteria from rhizospheric soils of Varanasi district.
2. Screening of K-solubilizing isolates on the basis of K-solubilizing capacity.
3. Physiological and biochemical characterization of K-solubilizing bacteria.
4. Periodical study on release of K from K-minerals by some virulent K-solubilizing bacterial isolates and
5. To study the production of growth promoting substances by K-solubilizing isolates and their effect on seed germination, growth and yield of maize.

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Increasing cost of the fertilizers with less nutrient use efficiency (NUE) necessitates alternate means to fertilizers. Soil is a storehouse of nutrients and energy for organisms living under soil-plant-microorganism system. These rhizospheric microorganisms are important component of sustainable agricultural ecosystems. They are involved in sustaining soil as well as crop productivity through organic matter decomposition, nutrient transformations and biological nutrient cycling. The rhizospheric microorganisms regulate the nutrient flow in the soil by assimilating nutrients and producing biomass and converting organically bound forms of nutrients. Soil microorganisms play an important role in various chemical transformations of soils and thus, influence the availability of macro and micro nutrients. Use of plant growth promoting microorganisms (PGPMs) helps in increasing yields in addition to conventional plant protection. Most important PGPMs are *Azospirillum*, *Azotobacter*, *Bacillus subtilis*, *B. mucilaginosus*, *B. edaphicus*, *B. circulans*, *Paenibacillus* spp., *Acidithiobacillus ferrooxidans*, *Pseudomonas*, *Burkholderia*, potassium (K), phosphorous (P), zinc (Zn) solubilizing microorganisms or SMART-microbes these are ecofriendly and environmentally safely.

Potassium solubilizing bacteria (KSB) are direct contributors to enhance the soil fertility in relation specially release of potassium from its fixed form to plant available form that directly influenced plant growth, development, yield and yield attributes, it's also enhance the plant resistance power against insect and pest. KSB also enhances nutrient uptake mainly potassium. So, KSB with different fertility levels of potassium maintain long-term soil sustainability, crop productivity and ecological sustainability with the improved economic condition of the country.

This chapter contains a comprehensive, up-to-date review of important research works pertaining to the topic “**Isolation, Characterization and Screening**

of Potassium-Solubilizing Bacteria from Soils of Varanasi” and has been described in subheadings as follows

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2.1 Importance and overview on KSB

2.1.1 Potassium and its importance

Potassium is an essential element for all living organisms. In plant physiology it is the most important cation in regard to its content in plant tissues and with respect to its physiological and biochemical functions (Zhang and Kong, 2014). The quantity of potassium absorbed by roots is second only to that of nitrogen for most cultivated

plants; however the demand for potassium is higher than that for nitrogen in banana, cotton and some other species (Schiavon *et al.*, 2010; Mora *et al.*, 2012). As the cation K^+ it is dissolved in the soil solution as well as adsorbed to clay and organic colloids. But it can also be part of more complex chemical compounds (Zandonadi *et al.*, 2010; Meena *et al.*, 2015).

Potassium is absorbed by roots and translocated inside the plant as the positive cation K^+ . It is characterized by a high mobility at all levels inside the plant in individual cells, tissues, and in long distance transport via the xylem and phloem, although no structural role of potassium has been found. This is in contrast to calcium and magnesium, which have important structural functions, but only limited mobility in plants. Control of plant water status is an important potassium function. It promotes water absorption by the roots, keep osmotic tension and turgor in cells and plant tissues and regulate the activity of stomata cells to prevent unnecessary water loss by transpiration. Potassium has a role in photosynthesis and in the production and translocation of carbohydrate to areas of meristematic growth, fruit development and storage.

The carbohydrate production and transport function is very important in vegetables and fruit production as it directly affects sugar and starch accumulation. Early stages of potassium deficiency are reflected only in yield decreases. This stage of potassium deficiency is called "*hidden hunger*" as no specific symptoms appear on the plants. As the intensity of the deficiency increases, symptoms consisting in yellowing and eventual necrosis of the border in older leaves do appear, beginning from the tip and progressing backward over the leaf. Because it is a mobile element, when the potassium deficiency occurs, the element is transferred from old leaves to the young growing points. A slowdown of the growth rate is also present at this level of potassium deficiency. Main internal consequences of potassium deficiency are general reductions of strength of plant structures, loss of vigor; slow down of carbohydrate transport, less resistance to low water availability and to diseases.

Potassium deficiency causes stunting of growth with shortening of internodes. Its deficiency leads to a reduction in photosynthesis, blackening of tubers in the case

of potato, tips or margin of lower leaves of legumes, maize, cotton, tobacco and small grains are either scorched or burnt (Ashley *et al.*, 2005; Bahadur *et al.*, 2014). Potassium plays an important role in the maintenance of cellular organizations in regulating permeability of cell membranes and keeping the protoplasm in a proper degree of hydration. It activates the enzymes in protein and carbohydrate metabolism and translocation of carbohydrates and imparts resistance to plants against fungal and bacterial disease. Potassium is an essential element for the development of chlorophyll. It plays an important role in photosynthesis, i.e., converting carbon dioxide and hydrogen into sugars, for translocation of sugars, starch formation and metabolic activities of plants. (www.ikisan.com).

Adequate quantities of potassium are thus essential for a crop to achieve its full yield potential and also for many aspects of product quality such as grain size and appearance, tuber size, oil content, dry matter and starch content, percentage sugar and fruit ripening and quality. Many of the functions of potash in the plant are related to physiological conditions and stress. These functions are diverse and include efficient nitrogen and water use, drought tolerance, frost resistance and resistance to pests and diseases. It is therefore not surprising that lack of plant-available potash in the soil results in weaker, less vigorous crops that suffer major penalties in difficult growing seasons. In years when growing conditions and yields are good the response to potash may be modest, especially for crops like cereals, but in adverse years its contribution to optimum yields will be substantial. Adequate potassium, thus provides some 'insurance' against adverse conditions in difficult growing seasons.

2.1.2 Global potassium demand

Potash fertilization started in the 19th century, when Justus v. Liebig discovered that plants need, in different proportions and quantities. During the past 40 years, world potassium consumption has increased 2.5 fold. Between 1960 and 2000, the world use of potassium fertilizers rose from 9 to 22 Mt and potassium fertilizer use accounts for ~ 16% of total fertilizer usage. Potash consumption in the developed countries has increased 1.25 fold in the past 40 years, while the demand in developing countries expanded 22 fold from 0.5 Mt in 1960 to 11.3 Mt in 2000. The world

production of potash had been increased up to 37 Mt as well as the price of potash \$470 /per tonns since 2011 (www.infomine.com). However, K fertilizer cost has not to stop enhance every year, this has led to increased cost of rice production and farmer's income should reduce. The world demand for fertilizer was forecast to increase by 4.8% to 170.4 million metric tons by 2010/11 (Heffer and Prudhomme, 2010).

2.1.3 Potassium status of soils in India

In India, nutrient removal continues to exceed 10 Mt of $N + P_2O_5 + K_2O$ every year. Clearly, expansion in fertilizer application (input) continues to fall short of nutrient removals (output) resulting in depletion of soil fertility and negative nutrient balance sheet. Out of 371 districts for which more than 11 million soil test data are available, 76 districts are in low, 190 in medium and 105 districts in the high category. Thus 21% districts are in low, 51% in medium and 28% percent in the high category of potassium fertility status (Table 2.1). All the low and medium K soils which constitute 72% of the total need K fertilization for optimum yield and balanced soil fertility (Ramamurthy and Bajaj, 1969; Hasan, 2002).

About 70 to 75% of the K absorbed is retained by leaves, straw and stover. The remainder is found in harvested portions such as grains, fruits, nuts, etc. Whenever the soil cannot adequately supply the K required to produce high yields, farmers must supplement soil reserves with fertilizer K. Improvements in both quantity and quality will add to export earnings. The information presented here is based on ~ 11 M soil samples made available by soil testing laboratories run by state departments of agriculture and the fertilizer industry. Though 11 M soil tests are not sufficient to comprehensively cover a country which has > 140 M ha cultivated land, they reflected changing K fertility status of soils in different parts of the country and provide some measure of the need for scientific use of K fertilizers (Fig. 2.1).

2.1.4 Potassium fixation in soil

In addition to releasing K, soil minerals can also fix K, significantly affecting K availability. This involves the adsorption of K ions on two sites in the inter layers

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of weathered sheet silicates, such as illite and vermiculite. The degree of K fixation in soils depends on the type of clay mineral and its charge density, moisture content, competing ions and soil pH. Montmorillonite, vermiculite and weathered micas are the major clay minerals that tend to fix K (Sparks, 1987). Additionally, soil wetting and drying also significantly affects the K fixation. The fixation process of K is relatively fast, whereas the release of fixed K is very slow due to the strong binding force between K and clay minerals (Oborn *et al.*, 2005). Whether a soil fixes or releases K highly depends on the K concentration in the soil solution (Maurya *et al.*, 2014). In addition to organic acids, the H^+ concentration in the soil solution (via soil pH) seems to play a key role in K release from clay minerals.

Table 2.1. Nutrient balance (NPK) under major states of India (adopted from Srinivasarao *et al.*, 2011)

States	Additions	Removal	Balance
Andhra Pradesh	411.3	708.6	-297.3
Karnataka	330.3	734.4	-404.0
Tamil Nadu	304.2	726.1	-421.9
Kerala	72.3	278.6	-206.3
Madhya Pradesh	168.9	943.9	-775.0
Gujarat	146.1	1137.4	-991.3
Rajasthan	20.9	1014.8	-993.9
Maharashtra	420.8	1482.9	-1062.1
Uttar Pradesh	863.1	1842.1	-979.0
Punjab	38.4	1022.8	-984.5
Haryana	16.6	669.1	-652.5
Assam	56.0	255.8	-199.8
Jharkhand	9.8	169.6	-159.9
Orissa	63.0	383.6	-320.6
West Bangal	304.4	972.8	-668.4
Total (15 states)	3226.1	12342.5	-9116.5

Optimization of soil pH may be a means of enhancing K release. For optimized K fertilizer management practices, it is crucial to understand the factors that regulate K release from soil non-exchangeable pool. Recent investigations have

raised awareness of the impact of K on the soil structure and its ability to capture water. It has been reported that the application of mineral K fertilizers enhances the water-holding capacity of soils and also improves the structural stability of sandy soil in particular (Holthusen *et al.*, 2010). Higher water retention plays a key role in securing the soil productivity in water-limited areas. Therefore, more information is needed in order to understand the effect of K fertilization on the soil's physical properties and soil water holding capacity (Fig. 2.1).

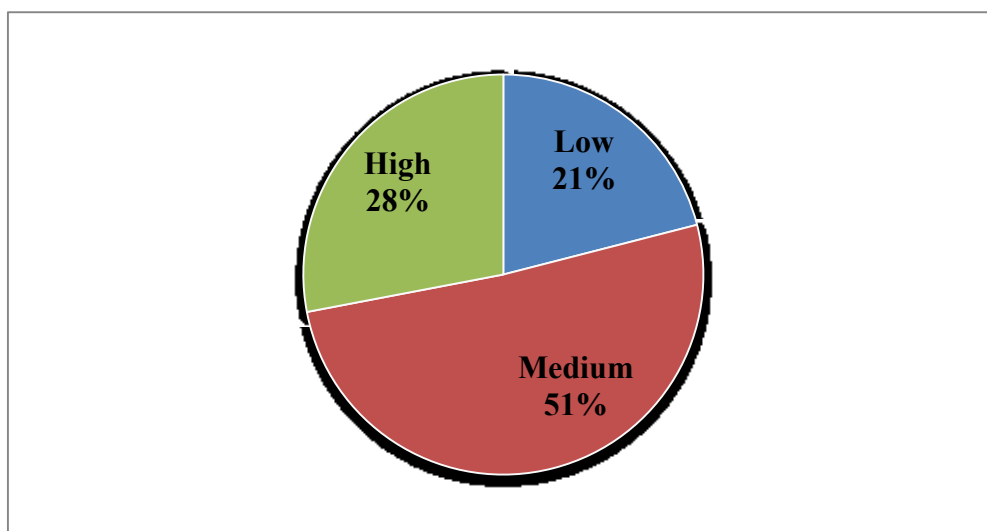


Fig. 2.1: The graphical representation of soil potassium status of India

2.2 Need for fertilization

2.2.1 Potassic fertilizers

The potassium content of potassic fertilizers is usually expressed as potassium oxide. K_2O , referred to as potash. These fertilizers are manufactured from minerals and ores. The commercial fertilizers are salts of potassium usually chlorides and sulphates which are soluble hence readily available to the plants. (www.incitecpivot.com).

The entire requirement of potash is met through imports as there are no exploitable reserves of potassic minerals in the country. The total import of MOP during the current year was 30.40 lakh MT (www.sobip.com). Studies also have shown that building up readily available potassium reserves in the soil ensures the

best opportunity for crops to achieve their optimum economic yield. Adding large amounts of potassium fertilizer to soil with little readily available potassium will not always increase yields to equal those in enriched soil. This is because in enriched soil, the potassium reserves are uniformly distributed throughout the layer of soil in which most of the roots grow. As potassium in the soil solution is depleted by crop uptake, it is rapidly replenished from the reserves. The benefits are often greater with those crops that have a short growing season. Such crops do not have very extensive root systems and they must acquire nutrients quickly to optimize growth. But the emergence of potassium fertilizers has not given the ultimate solution for developing countries like India because it lays a major economic constraint since large sums are spent on potassium fertilizers alone (Prabha, 2006).

2.2.3 Availability of potassium in soil

Only small amounts of K are maintained in the soil solution 5-20 kg/ha K (6-24 kg K₂O). The majority of potassium reserves in the soil are held by the negative charges on clay minerals and organic matter. The potassium may be held weakly or strongly according to the position in the clay lattice. K⁺ which is loosely held (exchangeable K) is rapidly released for plant uptake, whilst the more firmly bonded reserve ('non-exchangeable K') is released more slowly. Individual soils have different capacities to hold potassium according to clay type and content and amount of soil organic matter. Sandy soils have very limited reserves of exchangeable K. The clay minerals themselves contain potassium (Fig. 2.2)

2.2.4 Forms and availability of potassium

Potassium is the seventh most abundant element in the Earth's crust, and makes up ~ 2.4% by weight of the earth's crust. Yet only one to two per cent is available to plants. Potassium exists in the soil as dissolved K⁺ ions (solution K), exchangeable K, non exchangeable K and mineral K. Plants can take up solution K and exchangeable K from the soil, but the non exchangeable K, and mineral K are unavailable to plants. Depending on soil type, ~ 98% of total soil K is found in unavailable form. Most potassium minerals are insoluble. Feldspars and micas are

minerals that contain most of the K and common parent materials for most soils (Foth and Ellis, 1997). Plants cannot use the K in this crystalline (insoluble form). Over long periods of time (Fig. 2.2), these minerals weather (break down) and K is released, this process, however, is too slow to supply the full K needs of field crops.

2.3 Environmental factors affecting potassium solubilization

In soil, K mobilization is affected by many biotic and abiotic environmental factors like soil properties (physicochemical characteristics, aeration, and pH), presence of mycorrhizae fungi and rhizosphere bacteria and the composition of plant root exudates. These parameters may also influence the K mobilization ability of bacteria. Gahoonia *et al.* (1997) explained three hypothetical processes: (i) mineral fragmentation caused by the activity of root, increases the bacterial effect on mineral mobilization due to increased surface area for reactivity, (ii) root exudates help by indirectly providing the substrates for the production of weathering metabolites by bacteria, and (iii) besides producing weathering agents, bacteria produces phytohormones which stimulate the development of root, modify root exudation and physiology help improvement nutrient uptake and mobilization of minerals. Potassium concentration in various plants parts is not similar it's may varies from different climate conditions. In case of feldspar, illite and muscovite more K is released under aerobic condition as compared to anaerobic condition (Sheng, 2002; Badar, 2006). In liquid medium *Bacillus edaphicus* showed better growth and greater K releasing ability with illite as compared to feldspar (Sheng and He, 2006).

2.3.1 Potassium functions in plants

Potassium has many functions in plant growth, such as the smooth progress of cell division and growth, increases disease resistance and drought tolerance, regulates the opening and closing of stomata required for osmotic regulation. Besides, these potassium is essential for photosynthesis process and acts as key to activate enzymes to metabolize carbohydrates for the manufacture of amino acids and proteins. Furthermore, potassium assimilates transport during plant ontogeny and one of the most important influence is improvement in oil content of plants. Since the use of

potassium has covered a lot of plant activities, depletion in potassium uptake can cause problem for plant growth. Symptoms of potassium deficiency exhibit chlorosis along the leaf margins. Then, in severe cases, leaf will turn into yellow color and eventually will fall off. It also affects plant growth and canopy photosynthesis process. There are several factors that lead to this problem, for instance low soil potassium supplying capacity, insufficient application of mineral potassium fertilizer and biofertilizer, complete removal of plant straw, leaching losses and phosphorus and nitrogen deficiency (Das and Sen, 1981). Fundamentally, K^+ is highly water soluble and highly mobile and transported in the plants' xylem (Lack and Evans, 2005). Membrane transport of potassium can be mediated either by potassium channels, utilizing the membrane potential to facilitate transport of potassium down its electrochemical gradient, or by secondary transporters'. In plants, potassium act as regulator since it is constituted of ~ 60 different enzyme systems of drought tolerance and water use efficiency.

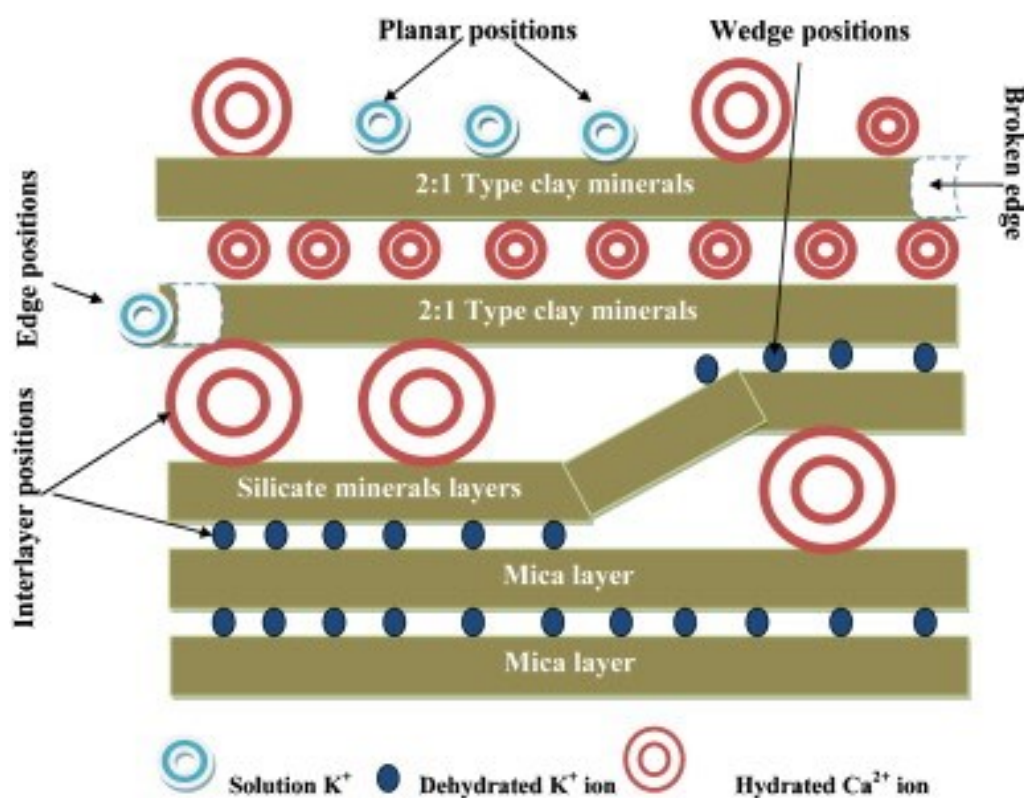


Fig. 2.2. Different potassium adsorption positions for K^+ in a mica-silicate minerals in soil system (this is modified figure of Rich, 1968)

Plants K concentrations of crops vary widely with site, year, crop species, and fertilizer input. It ranges from 0.4-4.3% (Askegaard *et al.*, 2004). According to Oborn *et al.* (2005) concluded that crop K concentrations are often well below (~ 3.5%). For many crops, the critical K concentration is in the range of 0.5 to 2% in dry matter (Leigh and Jones, 1984). With the exception of the cytosol and the vacuole, the sub cellular distribution of K is largely uncharacterized. The concentration of K in the cytoplasm is kept relatively constant at around 50-150 mM, while the concentration in the vacuole varies substantially depending on supply status. Together with accompanying anions (NO_3^- , Cl^- , malate^-), vacuolar K largely determines the osmotic potential of the cell sap. In the agronomic literature, high K concentration in crops have often been termed “*luxury consumption*” However, as outlined below “potassium nutrition and crop stress resistance”, high accumulation of K by crops during optimal growing conditions may be considered as an “*insurance strategy*” to enable the plant to better survive a sudden environmental stress (Kafkafi, 1990).

Plant species are known to differ in their K requirement and in their ability to take up K. The differences in absorption of K among different plant species are attributed to variations in root structure, such as root density, rooting depth and root hair length (Nieves-Cordones *et al.*, 2014). All crops required potassium, especially high carbohydrate plants such as maize, bananas and potatoes (Hillel, 2008). The KSB is a heterotrophic bacteria which obtain their energy and carbon from buildup of dead organic materials. Besides these KSB are aerobic bacteria which play an important role in maintaining soil structure by their contribution in the formation and stabilization of water-stable soil aggregates (WSA). In addition, this gram positive bacterium can produce a substance that stimulates plant growth or inhibit root pathogens (Egamberdiviya, 2006). Moreover, KSB specifically are well known for its capability to solubilize rock potassium mineral such as micas, illite and orthoclases. This is done through the production and excretion of organic acids (Maurya *et al.*, 2014; Subhashinia and Kumar, 2014). Therefore, KSB increases potassium availability in soils and increases mineral contents in plants.

2.3.2 Quantification of potassium solubilization

Studies on the dynamics of potassium solubilization by microorganisms are best carried out *in-vitro* based on the measurement of K released into culture broth from cultures developed using an insoluble mineral compound as the only K source. The rate of potassium solubilization is often estimated by subtracting the final K concentration (minus that of a non-inoculated control) from the initial theoretical K supplied by the K-bearing substrates. This estimate has the limitation of not taking into account the K utilized by the cells during growth. The efficiency of potassium solubilization by different microorganisms varies with the nature of potassium bearing minerals and more specifically it depends on the structure and chemical composition of minerals. It was observed that bacteria degraded kietyote and pegatolite and released 47 and 44.4 mg soluble potassium, respectively after 38 hours of incubation (Yakhontova *et al.*, 1987). The ease of release of potassium from minerals follow the order of Illite > feldspar > muscovite (Sheng *et al.*, 2002; Badar, 2006).

Besides, the releases of K from minerals also get affected by pH of the medium, dissolve oxygen and the microbial strain used. It was estimated that with the increase in pH from 6.5-to-8.0 the soluble K content increased from 490-to-758 mg/L. Instability of the potassium solubilizing character of some strains after several cycles of inoculation has been reported. However, the traits seem to remain stable in most isolates. Although no accurate quantitative comparison can be made from experiment with different sources of insoluble K, the review of literature suggests that fungi such as *Penicillium* spp., *Aspergillus* spp. are more effective solubilizers than bacteria such as *Bacillus* spp., *Paenibacillus* spp., *Pseudomonas* spp. etc.

2.4 Potassium solubilizing microorganism (KSMs)

A diverse group of soil micro-flora were reported to be involved in the solubilization of insoluble and fixed forms of K into available forms of K which is easily absorbed by plants (Zarjani *et al.*, 2013; Gundala *et al.*, 2013). Microbial inoculants that are able to dissolve K from mineral and rocks, that enhance plant growth and yield are also economically viable and ecofriendly. The first evidence of

microbial involvement in solubilization of rock potassium had shown by Muentz (1890). A wide range of KSMs namely *Bacillus mucilaginosus*, *B. edaphicus*, *B. circulans*, *Paenibacillus* spp., *Acidithiobacillus ferrooxidans*, *Pseudomonas*, *Burkholderia*, (Sheng *et al.*, 2008; Rajawat *et al.*, 2012; Liu *et al.*, 2012; Basak and Biswas, 2012) have been reported to release potassium in accessible form from K-bearing minerals in soils. Several fungal and bacterial species, popularly called as KSMs that assist plant growth by mobilization of insoluble forms of K. KSMs are ubiquitous whose numbers vary from soil to soil. Rhizosphere microorganisms contribute significantly in the solubilization of bound form of soil minerals in the soil (Supanjani *et al.*, 2006; Sindhu *et al.*, 2009). A variety of soil microorganisms have been found to solubilize silicate minerals (Sheng *et al.*, 2001).

Many microorganisms like bacteria, fungi, actinomycetes, mycorrhizae colonized even on the surface of mountain rocks (Groudev, 1987; Gundala *et al.*, 2013) have been reported that the silicate solubilizing bacteria *B. mucilaginosus* sub spp. *siliceus* liberates K from feldspar and aluminosilicates. According to Aleksandrov *et al.* (1967) that were isolated from agricultural land at different location and found that different bacterial species like silicate bacteria were found to dissolve K, silicates and aluminum from insoluble minerals, it also helps in the decomposition of organic matter, crop residues etc. and suggested that they play a major role in nutrient cycling in the soil-plant system. *B. mucilaginosus* stain CS1 is a silicates bacterium which exhibited inhibitory activity on the growth of gram negative bacteria. It has also been reported as silicate solubilizing bacteria present in rhizosphere as well as non-rhizosphere soil (Lin *et al.*, 2002). K-solubilizing bacteria were isolated from the roots of cereal crops which grown in potassium and silicate amended soil (Mikhailouskaya and Tchernysh, 2005).

2.4.1 Potassium solubilizing bacteria (KSB)

A wide range of rhizospheric microorganisms are reported as K-solubilizers include *B. mucilaginosus* (Zhao *et al.*, 2008; Basak and Biswas, 2009; Zarjani *et al.*, 2013), *B. edaphicus* and (Sheng, 2005), *B. circulans* (Lian *et al.*, 2002), *Burkholderia*, *Acidithiobacillus ferrooxidans*, *B. mucilaginosus* (Zhang and Kong,

2014), *B. edaphicus* (Sheng and He, 2006) *Arthrobacter* spp. (Zarjani *et al.*, 2013), *Enterobacter hormaechei* (Prajapati *et al.*, 2013); *Paenibacillus mucilaginosus* (Liu *et al.*, 2012), *P. frequentans*, *Clasdosporium* (Argelis *et al.*, 1993); *Aminobacter*, *Sphingomonas*, *Burkholderia* (Uroz *et al.*, 2007); *Paenibacillus glucanolyticus* (Sangeeth *et al.*, 2012). These microbial strains have the ability to solubilize K from K-bearing minerals, but only a few bacteria, such as *B. mucilaginosus* and *B. edaphicus*, have high activity in mobilizing and solubilizing of K from minerals (Sheng, 2005; Li *et al.*, 2006; Zhao *et al.*, 2008; Rajawat *et al.*, 2012). Bacteria have wide applications in mining, metallurgy, microbial fertilizer, and feed (Li *et al.*, 2006; Zhao *et al.*, 2008; Maurya *et al.*, 2014).

2.4.2 Potassium solubilizing fungi (KSF)

Arbuscular Mycorrhizal can increase the solubility of mineral form of potassium by releasing protons, H^+ or CO_2 and organic acid anions such as citrate, malate and oxalate. This also increase the nitrogen, potassium, calcium and iron in the plant leaves and fruits (Veresoglou *et al.*, 2011; Yousefi *et al.*, 2011). The inoculant of the two arbuscular mycorrhizal fungi (AMF) species, *G. mosseae* and *G. intraradices*, were applied in soil on a weight basis, recorded that the increasing the potassium uptake by maize crop (Wu *et al.*, 2005) Information on acquisition of the macronutrient cations by AM plants has been relatively inconsistent in that increases, no effects, and decreases have been reported (Clark and Zeto, 1996) in the case of potassium its depend on the soil condition as well as nature of plant growth and other condition (Clark and Zeto, 2000). Alves *et al.* (2010) reported that after 90 days, the plant height, shoot dry weight, root length, phosphorus and potassium contents, and mycorrhizae colonization were increased in comparison to control. Potassium acquisition compared to Ca and Mg was especially enhanced in AM switch grass grown in acid soil (Clark *et al.*, 1999). Ectomycorrhizal fungi, particularly isolate UFSC-Pt22 and UFSC-Pt186, contributed to the increase of the efficiency of alkaline breccias as a source of P and K to the plant growth of *Eucalyptus dunnii* seedlings, respectively (Alves *et al.*, 2010).

Prajapati *et al.* (2012) reported that potassium solubilizing fungi (KSF) strains such as *Aspergillus terreus* and *Aspergillus niger* were isolated from various K rich soil samples and observed *A. terreus* and *A. niger* which could solubilize insoluble potassium and showed the highest available potassium in liquid medium by using two various insoluble sources of potassium i.e. Feldspar and potassium aluminium silicate, based on their colonies and morphology characters. *A. terreus* shows highest K-solubilization as well as acid production on both the insoluble potassium source. The concentration of trace elements is another relevant factor in the context of rock solubilization by fungi (*A. niger*) also reported by production of acids (Mirminachi *et al.*, 2002). Furthermore, symbiotic nitrogen fixing rhizobia and *Pseudomonas*, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants, has also shown K and P-solubilizing activity. For instance, *Aspergillus* spp., *Aspergillus terreus* (Prajapati *et al.*, 2013), *Aspergillus niger* (Lian *et al.*, 2002; Prajapati *et al.*, 2012), *Penicillium* spp. (Sangeeth *et al.*, 2012) enhanced K-solubilization by mobilizing inorganic and organic K and release of structural K from rocks and minerals.

2.4.3 Mechanisms of K-solubilization

Currently, little information is available on K-solubilization by rhizospheric microorganism. Sheng and Huang (2002) found that K release from the minerals was affected by pH, oxygen and the bacterial strains used. The efficiency of the K-solubilization by different microorganisms was found to vary with the nature of potassium bearing minerals and aerobic conditions (Uroz *et al.*, 2009). The extent of potassium solubilization by *B. edaphicus* in the liquid media was more and better growth was observed on illite than feldspar (Sheng and He, 2006). Indigenous efficient rhizospheric microorganisms have the potential to absorb and mobilize the fixed form of potassium from trace mineral sources. Silicate bacteria were found to dissolve potassium, silica and aluminum from insoluble minerals. Hydrogen ion of soil or soil solution is directly related to releases of K from minerals. The content of potassium solubilization was enhanced 84.8-127.9% in microbial inoculated than uninoculated treatment. The extent of potassium solubilization of higher in illite by *B.*

edaphicus in the broth culture was reported as compared to feldspar (Sheng and He, 2006). Badar (2006) reported that the extent of potassium solubilization by silicate solubilizing, bacteria were recorded 4.90 mg/L at pH 6.5 to 8.0. *B. mucilaginosus* was recorded for 4.29 mg/L K-solubilization in media supplemented with muscovite mica (Sugumaran and Janarthanam, 2007).

Mechanism of potassium solubilization means by which insoluble potassium and structural unavailable form of potassium compounds are mobilized and solubilized due to the production of various type organic acids which accompanied by acidolysis, complexolysis exchange reactions and these are key processes attributed to the conversion in a soluble form (Uroz *et al.*, 2009). The organic and inorganic acids convert insoluble K (mica, muscovite, biotite feldspar) to the soluble form of K (soil solution form) with the net result increase the availability of the nutrients to the plants. The various types of organic acid produced by KSMs was differing with different organisms. Organic acids were detected in the microbial suspension (Zhang and Kong, 2014; Maurya *et al.*, 2014). KSMs have the ability to weather phlogopite via aluminium chelation and acidic dissolution of the crystal network (Abou-el-Seoud and Abdel-Megeed 2012; Meena *et al.*, 2014b).

The release of various types of organic acids were founded by microorganisms to solubilized the insoluble K to an available form of K which is easily uptakes by the plant. Researchers suggested that the plant growth promotion was related to K solubilization as well as the release of organic acids by the K-solubilizing strains. Sheng and He (2006) reported that solubilization of illite and feldspar by rhizospheric microorganisms is due to the production of organic acids like oxalic acid and tartaric acids, gluconic acid and 2-ketogluconic acid, oxalic acid, citric acid, malic acid, succinic acid, tarteric acid seems to be the most frequent agent of mineral K-solubilization (Prajapati and Modi, 2012; Zarjani *et al.*, 2013). Other organic acids, such as acetic, citric, lactic, propionic, glycolic, oxalic, malonic, succinic acid, fumaric, tartaric etc. have also been identified among K-solubilizers (Wu *et al.*, 2005).

The solubilization of structural K compounds by naturally-abundant KSMs is common under *in-vitro* conditions (Meena *et al.*, 2013; Zarjani *et al.*, 2013; Maurya *et*

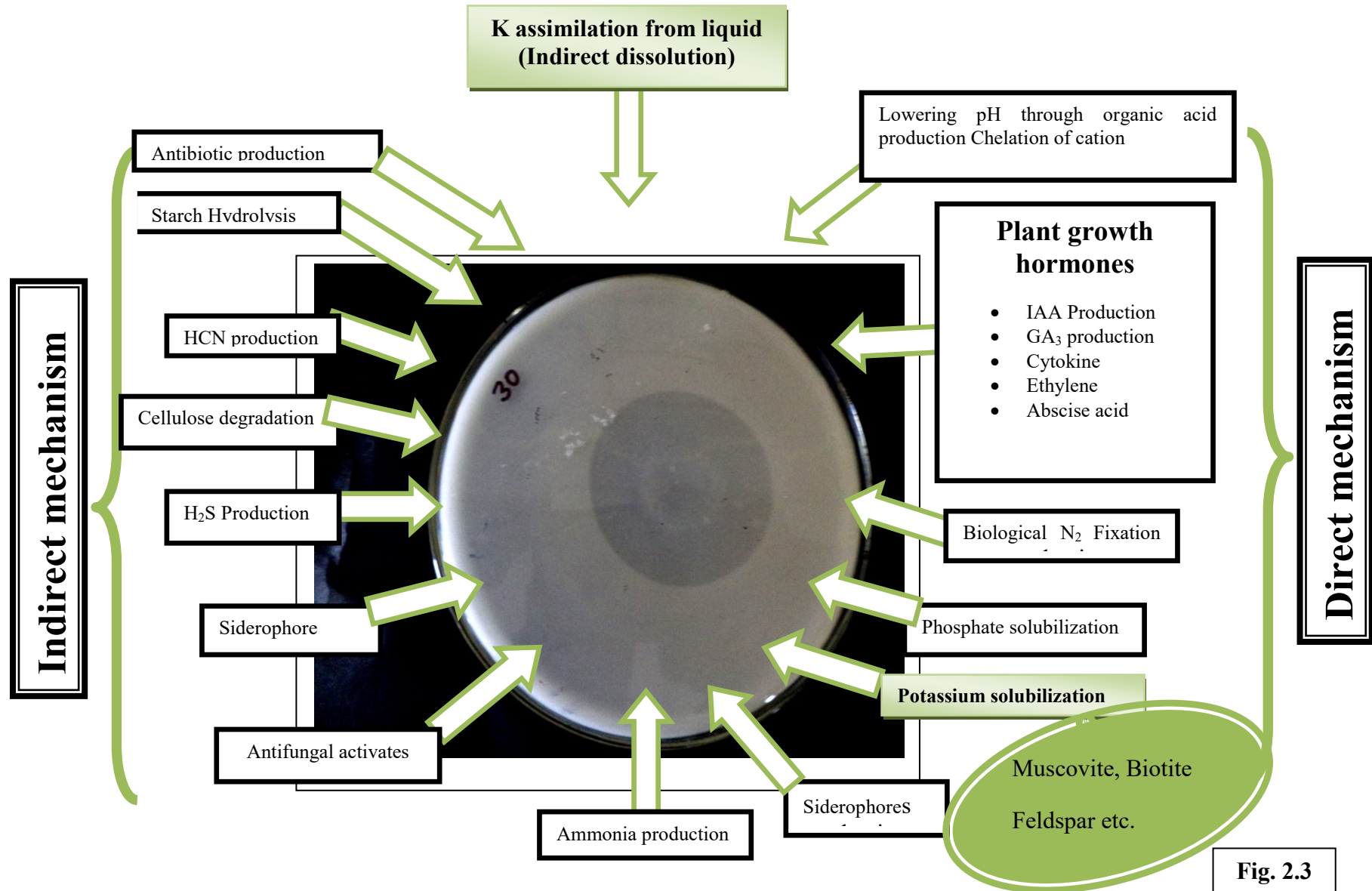
al., 2014), field and greenhouse condition (Singh *et al.*, 2010; Basak and Biswas, 2012; Prajapati *et al.*, 2013). Biomass of rhizospheric microorganism in the soil also contain a significant quantity of fixed K that is potentially available to plants (Subhashini and Kumar, 2014; Zhang and Kong, 2014).

Mechanisms for KSMs for solubilization of K are by: (i) lowering the pH, or (ii) by enhancing chelation of the cations bound to K and (iii) acidolysis of the surrounding area of microorganism. The lowering in pH of the medium suggests the release of organic acids and protons by the K-solubilizing microorganisms (Uroz *et al.*, 2009; Zarjani *et al.*, 2013). Such acidolysis by organic acids produced by the rhizospheric microorganisms can either directly dissolve the mineral K as a result of slow releases exchangeable K or readily available exchangeable K or can chelate both Si and Al ions associated with K mineral (Romheld and Kirkby, 2010). Thus, the synthesis and discharge of organic acids by the microorganisms into the surrounding environment acidify the microbe's cells and their surrounding environment that ultimately lead to the release of K ions from the mineral K by protonation and acidification (Goldstein, 1994). Of the different organic acids involved in the solubilization of insoluble K, succinic, citric, gluconic, α -ketogluconic and oxalic acids are the most prominent acids released by microbial strains (Table 2.2). The Fig. 2.3 showed the direct and indirect mechanisms of plant growth promoting properties of potassium solubilizing microorganism (KSMs) and their K-Solubilizing ability of mica (K- bearing mineral) on Aleksandrov medium.

Organic acids produced by KSMs can be detected by high performance liquid chromatography and enzymatic methods (Archana *et al.*, 2013; Zhang *et al.*, 2013). However, the acidification does not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral K (Rosa-Magri *et al.*, 2012; Zhang and Kong, 2014). Furthermore, the chelating ability of the organic acids is also important, as it has been shown that the addition of 0.05 M EDTA to the medium has the same solubilizing effect as inoculation with *Penicillium bilaii* (Sheng and He, 2006; Liu *et al.*, 2006).

Table 2.2. The categorization of districts as K-fertility status of India

States	Potassium status (kg K ₂ O/ha)		
	< 130	130-335	> 335
Andhra Pradesh	2	14	3
Arunachal Pradesh	2	3	0
Assam	7	3	0
Bihar and Jharkhand	1	24	2
Chandigarh	0	1	0
Dadra and Nagar Haveli	0	1	0
Delhi	0	1	0
Goa	1	0	0
Gujarat	0	3	16
Haryana	0	2	9
Himachal Pradesh	6	4	3
Jammu and Kashmir	5	5	0
Karnataka	3	10	7
Kerala	4	6	0
Madhya Pradesh and Chhattisgarh	3	10	31
Maharashtra	0	12	13
Manipur	1	0	0
Meghalaya	1	0	0
Mizoram	1	0	0
Nagaland	5	0	0
Orissa	2	11	0
Punjab	0	9	3
Pondicherry	1	0	0
Rajasthan	0	23	0
Sikkim	0	4	0
Tamil Nadu	0	6	7
Tripura	3	0	0
Uttar Pradesh and Uttaranchal	26	23	7
West Bengal	2	13	1
Total districts, (%)	76 (21)	190 (51)	105 (28)



2.5 Morphological and biochemical characterization

Cell morphologies of the KSB isolate were observed using an optical microscope after being stained with phenol red. Physiological tests, including Methyl red (MR) and Voges–Proskauer (VP) reactions, anaerobic growth, production of acid from carbohydrates, utilization of organic acids were determined by the method of Claus and Berkeley (1986). Subhashini and Kumar (2014) studied the substrate utilization patterns along with salt tolerance and temperature, pH and salt.

2.6 Molecular biology of potassium solubilizing microorganism

For maintaining turgid pressure of microbial and plant cells, potassium (K^+) is one of the important elements. Stimulation of potassium uptake is the most rapid response to an osmotic up-shock in bacteria. Potassium (K) is the most abundant intracellular cation, which has a major role in maintaining the turgor pressure of the cells and also play an important role in bacterial osmo-adaptation, pH regulation, gene expression, and activation of cellular enzymes (Epstein, 2003). Three different types of K transporters (Trk, Kdp, and Kup) have been involved for the uptake of K. Trk is a multi-component complex widespread in bacteria and archaea and it has a moderate affinity for the K uptake. Trk consist of a trans-membrane protein named TrkH or TrkG, which is the actual K-trans-locating subunit, and a cyto-plasmic membrane surface protein, TrkA, which is a NAD-binding protein required for the system's activity (Sleator and Hill, 2002). Kdp is an inducible system with high affinity and specificity for K, found in *Escherichia coli* and many other bacteria. Kdp is the only bacterial K uptake system whose expression is strongly regulated at the transcriptional level, which is mediated by the KdpD sensor kinase and the KdpE response regulator (Dominguez-Ferreras *et al.*, 2009). *Bacillus subtilis* has the Ktr gene which involve for K uptake. KT/KUP/HAK family, the genes of this family are homologous to bacterial KUP (TrkD) potassium transporters. The KUP transporter from *E. coli* is characterized by a midrange (0.37 mM) KM for K^+ and a similar affinity for Rb^+ and Cs^+ (Bossemeyer *et al.*, 1989).

Complementation of TK2463 cells by AtKUP1. The *E. coli* TK2463 mutant is defective in three K⁺ uptake transporters (Trk, Kup, and Kdp) (Epstein and Kim, 1971) and was transformed with plasmids containing the AtKUP1 gene or with an empty vector. The production of organic acids is considered as the principal mechanism for solubilization of mineral phosphate and potassium by microorganism, this assumption has been corroborated by the cloning of two genes involved in gluconic acid production viz., *pqq* and *gabY*. Gluconic acid is the principal organic acid produced by *Pseudomonas* spp., *Bacillus* spp., *Arthrobacter* spp., *Aspergillus* spp. and *Penicillium* spp. Other organic acids such as lactic, isovaleric, isobutyric, acetic, glycolic, oxalic, malonic and succinic, citric, acetic, tartaric, succinic acids are also generated by the different potassium solubilizing and phosphate solubilizing microorganism. The chelating substances and inorganic acids such as sulphidic, nitric, and carbonic acid are considered as other mechanisms for potassium and phosphate solubilization. Xiufang *et al.* (2006) identified and characterized potassium solubilizing bacteria by 16S rDNA gene sequencing analysis using universal primers, the forward primer and the reverse primer, the recombinant plasmids were developed from the cloned products and the relatedness between the strains was estimated from phylogenetic analysis (Xiufang *et al.*, 2006).

2.7 Effect of potassium solubilizing microorganisms (KSMs) on plant growth and yield

Inoculation of seeds and seedling treatment of plants with KSMs generally showed significant enhancement of germination percentage, seedling vigor, plant growth, yield and K uptake by plants in particular, under glasshouse conditions (Singh *et al.*, 2010; Awasthi *et al.*, 2011; Basak and Biswas, 2012; Zhang *et al.*, 2013; Zhang and Kong, 2014). The application of organo minerals with combination of silicate bacteria for enhancing plant growth and yield of maize and wheat was first reported by Aleksandrov (1958). More importantly, research investigation conducted under field level test crops such as wheat, forage crop, maize and sudan grass crops has revealed that KSMs could drastically reduce the usage of chemical or organic fertilizers (Xie, 1998). As reported by

previous researchers (Sindhu *et al.*, 2012; Zeng *et al.*, 2012) the enhancement of plant K nutrition might be due to the stimulation of root growth or the elongation of root hairs by specific microorganisms, thus no direct increase in the availability of soil solution K is expected.

KSMs have been isolated from rhizospheric soil of various plants and from K-bearing mineral (Zhang *et al.*, 2013), feldspar (Sheng *et al.* (2008), potato-soybean cropping sequence (Biswas, 2011), Iranian soils (Zarjani *et al.*, 2013), ceramic industry soil (Prajapati and Modi, 2012), mica core of Andhra Pradesh (Gundala *et al.*, 2013), common bean (Kumar *et al.*, 2012), biofertilizers (Zakaria, 2009), sorghum, maize, bajra, chilli (Archana *et al.*, 2013), cotton, tomato, soybean, groundnut and banana (Archana *et al.*, 2012), soil of Tianmu Mountain, Zhejiang Province (China) (Hu *et al.*, 2006), rice (Muralikannan, 1996), tea (Bagyalakshmi *et al.*, 2012), valencia orange (Shaaban *et al.*, 2012), black pepper (Sangeeth *et al.*, 2012), potato (Abdel-Salam and Shams, 2012), Thyme (Yadegari *et al.*, 2012), eggplant (Han and Lee, 2005) peanut and sesam (Youssef *et al.*, 2010), tobacco (Zhang and Kong, 2014). Better crop performance was reported to be achieved from several horticultural plants, vegetables and cereals, which were successfully inoculated with KSMs (Singh *et al.*, 2010; Basak and Biswas, 2012; Prajapati *et al.*, 2013). Potassium use efficiency (PUE) in agricultural lands could effectively be improved through the inoculation of relevant KSMs, which is in fact, an integration and sustainable mean of nutrient management of crop production. Enhancement of plant growth by improving N-fixer, P and K-solubilizers is another beneficial effect of microorganisms with K-solubilizing potential (Maurya *et al.*, 2014; Zhang and Kong, 2014). A hydroponics study was carried out by Singh *et al.* (2010) to evaluate the effect of *Bacillus mucilaginosus*, *Azotobacter chroococcum*, and *Rhizobium* spp. on their ability to mobilize K from waste mica using maize and wheat as the test crops under a phytotron growth chamber. The significant K assimilation was recorded in maize and wheat, where waste mica was the sole source of K; this was translated into higher biomass accumulation, K content and uptake by plants as well as chlorophyll and

crude protein content in plant tissue. Among the rhizobacteria, *B. mucilaginosus* resulted in significantly higher mobilization of potassium than *Azotobacter chroococcum* and *Rhizobium* inoculation. According to Sheng and He (2006), investigation of K mobilization by the wild-type strain NBT of *B. edaphicus*, in a pot experiment, wheat was grown in a yellow-brown soil that had low available K. After inoculation with bacterial strains, the root growth and shoot growth of wheat were significantly increased and higher NPK contents of plant components as compared to un-inoculated.

Inoculation with KSMs have been reported to exert beneficial effects on growth of cotton and rape (Sheng, 2005), pepper and cucumber (Han *et al.*, 2006), khella (Hassan *et al.*, 2010), sorghum (Badar *et al.*, 2006), wheat (Sheng and He, 2006), tomato (Lin *et al.*, 2002), chilli (Ramarethinam *et al.*, 2005) and sudan grass (Basak and Biswas, 2009; Basak and Biswas, 2010), tobacco (Zhang and Kong, 2014). Similarly, Zahra *et al.* (1984) reported that the effect of soil inoculation of the silicate bacteria *B. cirulans* for solubilization of K and Si from different minerals and soil showed significant increase of organic matter and ~ 17% yield of rice (Muralikannan, 1996). Increased wheat yield up to 1.04 t/ha reported by Mikhailouskaya and Tcherhysh (2005) with inoculation of KSMs on several eroded soils which are comparable with yields on moderately eroded soil without bacterial inoculation and dry matter production also increased. According to Badar *et al.* (2006), the co-inoculation of KSMs with K and P bearing minerals on sorghum were recorded to enhanced dry matter yield (48, 65 and 58%), P (71, 110, and 116%) and K (41, 93 and 79%) uptake in three different soils clay, sandy and calcareous soils, respectively. Archana *et al.* (2008) reported that the KSMs was isolated from rock and rhizosphere soils of greengram (*Vigna radiata*) and reported that these KSMs enhanced the solubilization of K in acid leached soil as well as increased seedling growth and yield of greengram. Sugumaran and Janarthanam (2007) reported that increased in the dry matter by 25% and oil content 35.4% of groundnut crop and available P and K increased from 6.24 and 9.28 mg/kg and 86.57 to 99.60 mg/kg, respectively in soil due to inoculation of *B. mucilaginosus* as compare to un-inoculated control.

According to Archana *et al.* (2012) the efficient K solubilizing bacteria *Bacillus* spp. showed increase in growth and yield of maize. It indicates that the KSMs significantly increased plant growth, nutrient uptake and yield component over absolute fertilizer control. Supanjani *et al.* (2006) reported that integration of P and K rocks with inoculation of P and K- solubilizing bacteria increased P availability from 12-to-21% and K availability from 13-to-15%, respectively. Soil application of KSMs, plant has ~ 16% photosynthesis and 35% higher leaf area in comparasion to control. The overall results of this finding is the treatment of P and K rocks with P and K solubilizing bacterial strain were sustainable alternative of chemical fertilizer for crop production.

K-solubilizing bacteria are extensively used as biofertilizers in Korea and China as significant areas of cultivated soils in these countries are deficient in plant-available K is considered to be a major limiting factor for food production in many agricultural soils (Xie, 1998). Thus the application of K-solubilizing bacteria as biofertilizers for agriculture improvement can reduce the use of agrochemicals and support ecofriendly crop production (Prajapati *et al.*, 2013; Maurya *et al.*, 2014; Zhang and Kong, 2014). Therefore, it is imperative to isolate more species of mineral-solubilizing bacteria to enrich the pool of microbial species and genes as microbial fertilizers, which will be of great beneficial to the ecological development of agriculture (Liu *et al.*, 2012).

2.8 Future prospect of potassium solubilizing microorganisms

Potassium solubilizing microorganisms play an important role in plant nutrition through increase in K uptake and promotion of growth and yield of crops. Investigation is needed to improve the performance and use of potassium solubilizing microorganism as microbial inoculants. Greater attention should be paid to studies and application of new combinations of potassium solubilizing and other plant growth promoting microorganisms. The mechanisms explaining the synergistic interaction should be a matter of research to elucidate the biochemical basis of these interactions. On the other hand, genetic manipulation of potassium-solubilizing microorganism to improve their

potassium solubilizing ability/capabilities and/or the introduction of this trait in strains with other plant growth promoting effects is not only important, but also seems to be practically feasible. In addition, the selection by classical genetic methods of mutants with increased production of organic acids could constitute an effective approach that cannot be underestimated. Genetic manipulation by recombinant DNA technology seems to offer a feasible approach for obtaining efficient KSB strains. Cloning of genes involved in mineral potassium solubilization, such as those influencing the synthesis of organic acids would be the first step in such a genetic manipulation program.

Future research should also investigate the stability and performance of the potassium solubilization trait once the bacteria have been inoculated in soil, in both natural and genetically modified strains. The survival and establishment of the introduced strain can be affected by low competitiveness, thus limiting the effectiveness of application. On the other hand, the putative risk involved in the release of genetically engineered microorganisms in soil is a matter of controversy, in particular with regard to the possibility of horizontal transfer of the inserted DNA to other soil microorganisms. For these reasons, the use of genetic reporter systems, such as bioluminescence genes, or green fluorescent protein genes is crucial in studying the fate and survival of the strain in soil. Genetic engineering of the potassium solubilizing character must eventually be directed to the chromosomal integration of the gene for higher stability of the character and to avoid horizontal transfer of the inserted gene in soil. This strategy would also prevent the risk of metabolic load caused by the presence of the plasmid in the bacterial cell. On the other hand, chromosomal integration may have the disadvantage of a low expression of the activity, due to the low copy number of the gene, in comparison with plasmid-harbored genes. An alternative to this situation might be the integration of multicopies of the target gene. Additionally, the use of powerful and species-specific promoters, which could be activated under the specific environmental conditions of soil, is another interesting approach to successful gene expression in the engineered strain.

MATERIALS AND METHODS

The present investigation entitled “**Isolation, Characterization and Screening of Potassium-Solubilizing Bacteria from Soils of Varanasi**” involved an *in-vitro* and *in-vivo* experiments conducted at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The bacterial colonies were identified based on their plaque formation. Morphological characteristics of isolates were examined and their ability to solubilize potassium from insoluble waste mica was studied. Molecular characterization of some promising isolates was done. The materials used, procedures and techniques adopted are given in detailed in this chapter.

3.1 General

3.1.1 Location

All the laboratory experiments were conducted in Soil Microbiology Laboratory, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi which is located at an altitude of 80.71 M above MSL, and at 25°18' N latitude and 80° 36' E longitudes.

3.1.4 Sterilization

All the required media and water were sterilized at 15 lbs pressure for 20 min in autoclave and the glass wares were sterilized at 160°C for 3 h in hot air oven.

3.1.5 Collection of rhizospheric soil samples

The rhizospheric soils from different crops were collected from soils of eight blocks *Arajiline*, *Baragaon*, *Chiraigaon*, *Cholapur*, *Harhua*, *Kashi Vidya Peeth*, *Pindara*, and *Sewapuri* of Varanasi district for study. Details of places and crops of soil sample (Table 3.1).

Soil samples were taken from root attached soils of plants. Some top soil was removed before the collection of soil samples. Surface soil was digged to ~ 10 cm soil layer around the plant with the help of the khurpi, where roots of crops were concentrated. From ~ 0 to 2.5 mm away from the root surface, a zone of soil is located that is significantly influenced by living roots and is referred to as the rhizosphere. The rhizosphere soil and roots were separated from the bulk of the soil by hand. A total 94 soil (forty five plants) samples were taken randomly in every block of Varanasi District and ~ 20 g of rhizosphere soil was collected around each crop plants. All soil samples were sealed in sterilized ziplock bags, stored in an ice chest, and used within 10-14 h of collection. A total ninety four rhizospheric soil samples were collected from different field crop in rainy season June to August 2012 which is the peak growing season for kharif crops. Rhizosphere soil samples were collected in rainy season (June to August).

3.1.6 Waste mica

Waste mica (WM), a K-bearing mineral was obtained from the surroundings of mica mines located at Koderma district of Jharkhand, India that generates during the dressing of raw mica blocks. It was ground in a Wiley mill to 2 mm size. Ground mica was analyzed for its chemical characteristics following the standard procedures (Table 3.2 and Fig. 3.1). The sieved powder was submerged in sterilized water for 48 hr to get soluble K or plant available K.

3.1.7 Plating method

Potassium solubilizing bacteria (KSB) were isolated and enumerated from collected soil samples by serial dilution plate count method using Modified Aleksandrov Medium (MAMs).

3.1.8 Preparation of serial soil dilution

Weighted 10 g of the test soil sample under aseptic condition on a sterile paper and transferred it to 90 mL of sterile saline water, shaken thoroughly for uniform mixing

Materials and Methods

and from this suspension, with the help of a sterile pipette, 1 mL of suspension was transferred into culture tube containing 9 mL of sterile distilled water. Shaken the culture tube thoroughly and from this, further dilutions were made by transferring 1 to 9 mL of sterile saline water aseptically up to a dilution level of 10^{-4} . From this suspension, 1 mL was poured and spread on medium plate containing waste mica with the help of sterile pipette and glass triangle in the aseptic condition. Plates for each soil were preprated and incubated in BOD at $28 \pm 2^{\circ}\text{C}$ temperature for seven days.

Table 3.1: Block wise sampling site of Varanasi district with crops

Block name	No. of samples	Rhizospheric crops
Arazline	11	<i>Zea mays</i> , <i>Cajanus cajan</i> , <i>Nicotiana tabacum</i> , <i>Saccharum officinarum</i>
Bada Ganv	10	<i>Zea mays</i> , <i>Solanum tuberosum</i> , <i>Cajanus cajan</i> , <i>Musa paradisiaca</i> , <i>Saccharum officinarum</i>
Chiraigaon	12	<i>Zea mays</i> , <i>Nicotiana tabacum</i> , <i>Saccharum officinarum</i>
Cholapur	10	<i>Zea mays</i> , <i>Saccharum officinarum</i> , <i>Musa paradisiaca</i> , <i>Cajanus cajan</i>
Harhua	11	<i>Zea mays</i> , <i>Musa paradisiaca</i> , <i>Cajanus cajan</i> , <i>Solanum tuberosum</i> , <i>Saccharum officinarum</i> , <i>Nicotiana tabacum</i>
Khasi Vidyapeeth	13	<i>Zea mays</i> , <i>Solanum tuberosum</i> , <i>Cajanus cajan</i>
Pindra	14	<i>Zea mays</i> , <i>Musa paradisiacal</i> , <i>Solanum tuberosum</i> , <i>Nicotiana tabacum</i> , <i>Saccharum narum</i>
Sewapuri	13	<i>Zea mays</i> , <i>Cajanus cajan</i> , <i>Saccharum officinarum</i> , <i>Saccharum officinarum</i> , <i>Nicotiana tabacum</i>
Total samples	94	

Table 3.2: Elemental composition of waste mica (muscovite and biotite)

Minerals	Silica	Iron	Soluble potash	Magnesium Oxide	Sodium Oxide	Manganese oxide	Phosphorus	Calcium Oxide
	Si ₂ O ₃ (%)	Fe ₂ O ₃ (%)	K (%)	MgO (%)	Na ₂ O (%)	MnO (%)	P (%)	CaO (%)
Muscovite	45.10	2.54	9.82	0.61	0.37	Traces	0.022	0.23
Biotite	38.42	16.24	9.70	11.94	0.24	Traces	0.019	0.14

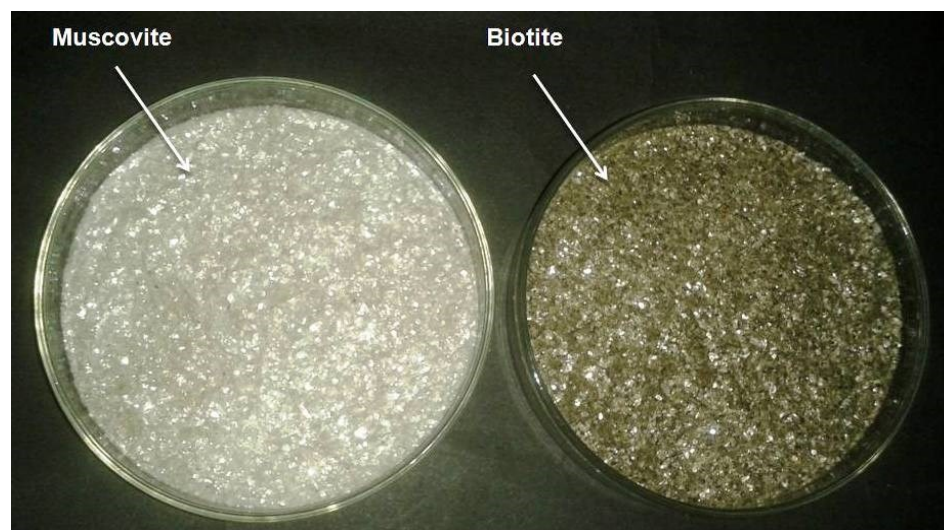


Fig. 3.1: Waste mica (muscovite and biotite) used during the investigation, after processing in Wiley mill and sieved through 2 mm sieve (material collected from the surroundings of mica mines located at Koderma district of Jharkhand, India).

3.1.9 Enumeration of K-solubilizing bacteria

Bacterial colonies forming plaque around them were counted. Population was expressed in g⁻¹ oven dried soil. Colony forming unit (CFU) in the plates for each soil were counted and averaged.

3.2 Isolation of K-solubilizing bacteria

Modified Aleksandrov medium (MAMs) (WM powder was added to the Aleksandrov medium as the sole source of K) and used to screen K-solubilizing bacteria in the rhizospheric soil. The serially diluted soil samples were plated on Aleksandrov medium containing 5 g glucose, 0.005 g MgSO₄·7H₂O, 0.1 g Fe Cl₃, 2.0 g CaCO₃, 3.0 g waste mica (muscovite and biotite) as a potassium mineral (2.0 g was used in original media), 2.0 g calcium phosphate and 20 g agar- agar (Hu *et al.*, 2006). The plates were then incubated at 28 ± 2°C. After 7 days of incubation colonies showing clear zone of formation around their colonies were considered to be the KSB and selected for further studies using Aleksandrov broth (Sugumaran and Janartham, 2007). The zone of solubilization of purified K-solubilizing bacterial isolates were measured using scale after 7 days of plating on Aleksandrov medium. Morphological characteristics of KSB isolates were studied using following standard techniques (Holt *et al.*, 1994). The diameter of the solubilization zone was measured in cm and the values were reported as mean ± standard error for each sample. Screened strains were transferred to a slant with modified solid Aleksandrov medium and maintained at low temperature in refrigerator at 4°C.

3.2.1 Characterization of potassium solubilizing bacteria

3.2.1.1 Morphological characterizations

a. Gram Staining and Microscopic Examination (Rangaswami, 1975)

Gram staining reagents:- Crystal violet (primary stain), Iodine solution (Mordant), Alcohol (decolorizing agent), Safranin (counter stain). Grams staining of the inoculants

were carried out as per Hucker's modified method (Rangaswami, 1975). A drop of sterilized distilled water was taken on the middle of each clear and flamed slide. Then bacterial growth was transferred to the sterilized drop of water and a very thin smear was prepared on each slide by spreading uniformly. The film was fixed by passing it over the gentle flame for two or three times. The slides were flooded with crystal violet solution and allowed to stand for 1 minute and then washed thoroughly with gentle stream of tap water. The slides were then immersed in iodine solution for one minute and washed thoroughly with 95% alcohol till release of blue colour finished.

Excess alcohol was drained off and washed thoroughly with gentle stream of tap water. The slides were then covered with safranin for five minutes. The slides were washed with sterilized water and air dried. The cell morphology of inoculants was observed under microscope. The bacteria which retain the primary stain (appear dark blue or violet) are called gram-positive, whereas those that lose the crystal violet and retain counter stained of safranin (appear red) are referred as gram-negative.

b. Colony morphology (Gerhardt *et al.*, 1981)

Colony morphological characteristics of selected KSB strain were studied on the basis of their colony characters such as elevation, colour, margin, optical density and size of plaque.

c. Zone of solubilization (Aneja, 2003)

Diameter of zone of solubilization was recorded at 10 DAI and measured with the help of scale and expressed in centimeter.

d. Slime production (Aneja, 2003)

After 10 days of incubation the plates ($28 \pm 2^\circ\text{C}$), levels of slime production was examined. For this colonies were touched with a red hot inoculation needle and up lifted. Length of the threat formed was experienced as low, medium and high level of slime production by the isolates.

e. Motility Test (Health Protection Agency, 2007)

The motility test medium is a semi-solid agar designed to demonstrate motility by diffusion. This method is to determine if the organism are motile by means of flagellum. Non-motile bacteria do not produce flagella. The KSB motility was observed from examination of the tubes following incubation. Growth spreads out from line of inoculation if the KSB was motile. Highly motile KSB provides growth throughout the tube. Growth of non-motile KSB only occurs along the stab line if the bacteria was motile they will diffuse into the soft medium laterally from the line of inoculation. Non-motile KSB grow along the line of inoculation only.

3.3 Biochemical characterization of potassium solubilizing bacteria

For the biochemical characterization of KSB done by following the slandered procedure (Table 3.3).

3.4 Physiological characterization of KSB

a. Temperature tolerance

Bacterial growth at different temperature was determined in Aleksandrov broth at temperatures 20, 25, 30, 35, 40 and 45°C. The optical density (OD) was measured at 420 nm at 14 days of incubation.

b. pH tolerance

Tolerance to pH levels 6.4, 7.0, 7.6, 8.2, 8.8 and 9.4 were tested in Aleksandrov broth. NaOH was used to adjust pH above 7. 100 µl of exponential phase culture was inoculated in 30 mL Aleksandrov broth and incubated at $28 \pm 1^\circ\text{C}$. Optical density was measured at 420 nm after 14 days of incubation.

c. Salt tolerance

Tolerance to sodium chloride (NaCl) was determined in Aleksandrov broth supplemented with 1, 2, 4, 6, 8 and 10% NaCl. After 14 days of incubation at $28 \pm 1^\circ\text{C}$ optical density was measured at 420 nm.

Table 3.3: Procedure followed for biochemical characterization of potassium solubilizing bacteria

Test parameter	References
Biochemical analysis	
Starch Hydrolysis Test	Seeley and Van demark, 1981
Hydrogen Sulfide (H ₂ S)	Aneja, 2003
Catalase test	Smibert and Krieg, 1981
Acid Production	Xiufang <i>et al.</i> 2006
Urease Test	Christensen, 1946
Fermentation of carbohydrates	Seeley and Van demark, 1981
IMViC Tests	
Indole Production Test	Gillus, 1956
Methyl Red- Voges Proskauer Test	Omeara, 1931
Citrate Utilization Test	Simmons, 1976

3.5 Plant growth promoting attributes of isolates

Bacterial strains were screened for plant growth promoting attributes by assessing the production of ammonia, hydrogen cyanide (HCN), gibberellic acid and indole-3-acetic acid (IAA) production.

3.5.1 Production of Indole-3-acetic acid

Indole-3-acetic acid (IAA) production was detected by the modified method as described by Brick *et al.* (1991). Quantitative analysis of IAA was performed using the method of Loper and Scroth (1986) at different concentrations of tryptophan (150 mg mL⁻¹). KSB cultures were grown for 48 h on their MAMs at 28 ± 2°C. Fully grown cultures were centrifuged at 3000 RPM for 30 min. The supernatant (2 mL) was mixed with two drops of ortho-phosphoric acid and 4 mL of the Salkowski reagent (50 mL, 35% of Perchloric acid, 1 mL 0.5 M FeCl₃ solution). Optical density of pink color was taken at 530 nm with the help of spectrophotometer. Concentration of IAA produced by cultures

was measured with the help of standard graph of IAA (Hi-media) obtained in the range of 10–100 mg/mL.

3.5.2 Production of ammonia and gibberelic acid

To assess the ammonia production, freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated at $37 \pm 2^\circ\text{C}$ for 24–72 h, after then Nessler's reagent (0.5 mL) was added in each tube. Development of brown to yellow color indicated positive for ammonia production (Cappuccino and Sherman, 1992). An individual colony of test strain was streaked on NA plates supplemented with 4.4 g glycine L^{-1} , to screen for cyanide production. Thereafter, a piece of filter paper (Whatman filter paper no. 1) impregnated with 0.5% picric acid (yellow) and 2.0% sodium carbonate was placed in the lid of each Petri dish. The Petri dishes were sealed with parafilm and kept at 28°C for 48–96 h. Discoloration of the filter paper orange to brown after incubation indicates microbial production of cyanide (Bakker and Schippers, 1987). Gibberelic acid production measured according Paleg (1965).

3.6 Potassium solubilization ability of KSB

The waste mica powder was added to the liquid Aleksandrov medium as the sole source of K to test the ability of the isolates for K -solubilization. To analyze the ability of K-mineralizing bacteria in liquid Aleksandrov medium, 50 mL of Aleksandrov solution was added into 250 mL conical flasks and autoclaved. After cooling 1 mL of K-solubilizing bacterial culture raised to a population of 10^8 by overnight shaking was inoculated. The uninoculated Aleksandrov solution was used as a control. Three flasks (under each isolate) were incubated at 150 RPM and $28 \pm 1^\circ\text{C}$ for 7, 14 and 21 days. Three sets were prepared for different incubation periods for the quantitative measurement of available K content.

3.7 Periodical changes in pH and EC of broth

The isolates were inoculated in broth separately. At each incubation period broths was filtered through Whatman No.1 filter paper and filtrate was taken in the 50 mL beaker. The pH and EC of broth for different KSB strains were measured at 7, 14, and 28 DAI with the help of digital pH meter (Chopra and Kanwar, 1982) and by using digital EC meter (Sparks *et al.*, 1996).

3.8 Quantification of K

The incubated broth containing K-solubilizing bacteria (50 mL) were vortexed for 10 min to eliminate insoluble materials. Fifty milliliters broth cultures were centrifuged at 10,000 RPM for 10 minutes in the REMI-centrifuge machine to separate the supernatant from the cell growth and insoluble waste mica (muscovite and biotite). Potassium content in the supernatant solution of culture was determined by flame photometry and compared with uninoculated control (Sugumaran and Janarthanam, 2007). This experiment was repeated three times.

3.8.1 Preparation of KCl solution standard curve

Potassium chloride was dried at 60°C and 1.908 g was dissolved in distilled water and made up the volume to 1 L. This gives standard solution of 1000 ppm. From this standard solution 10 mL was diluted to get 100 mL of working standards. From this working standard solution 10, 20, 30, 40 mL was transferred into 100 mL volumetric flask and volume was made up by distilled water up to the mark. Thus, 0, 10, 20, 30 and 40 ppm solution of K was prepared and used for the preparation of the standard curve.

3.9 Amplification of 16 S rDNA genes by polymerase chain reaction

Universal primer was used for the amplification of 16S rDNA gene in all bacterial isolates. This primer was custom synthesized by Bangalore Genei, Bangalore, India. The 50 µl of reaction mixture consisted of 50 ng of genomic DNA, 2.5 U of Taq polymerase,

5 µl of 10 X buffer (100 mM Tris-HCl, 500 mM KCl pH-8.3), 200µM dNTP, 1.5mM MgCl₂ and 10 pmoles of each primer. The forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-TACGGTTACCTTGTTACGACTT-3') were used (Narde *et al.*, 2004). Amplification was performed under the following PCR (PCR System 2720, Applied Biosystems, Singapore) conditions: initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1.5 min, extension at 72°C for 2 min and a final extension at 72°C for 7 min.

Amplified PCR products (5 µl) were resolved on a 1.5% (w/v) agarose gel at 100 V for 45 min in 1X TAE buffer containing ethidium bromide (EtBr) along with 500 bp DNA ladder (Bangalore Genei Pvt., Ltd. Bangalore, India). PCR product size was observed approximate 1500 bp. The PCR product was purified using a PCR purification kit (Bangalore Genei, Bangalore, India) for the sequencing of 16S rDNA.

3.9.1 DNA Sequencing

Sequencing of 16S rDNA was carried out at Bangalore Genei Pvt. Ltd., Bangalore, India. The 16S rDNA sequences were analyzed with the nucleotide database available at the GenBank using the BLAST tool at NCBI (www.ncbi.nlm.nih.gov) for identification of bacteria and were submitted at NCBI Gen Bank and also prepared Phylogenetic relationships among 16S rDNA.

3.10 Pot experiment

3.10.2 Climate

Varanasi falls in a semi-arid to sub-humid climate with moisture deficit index between 20 to 40. The normal period for the onset of monsoon in this region is the 3rd week of June which lasts up to end to September or sometimes extends up to the first week of October. Showers of rain are experienced during winter season. The annual rainfall of this region is ~ 1100 mm. Generally the maximum and minimum temperature

ranges between 20-42°C and 9-28°C respectively. May and June are the hottest month with a mean maximum temperature ranging from 39 to 42°C. The 4 cold period lies between November to January with minimum temperature varying between 9-10°C. The mean relative humidity is about 68% which rise to 82% during wet season and goes down to 30% during dry season.

3.10.3 Experimental soil

The Alluvial soil sample was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Initial properties of soil is presented below (Table 3.4).

3.10.4 Seed treatment with potassium solubilizing bacteria

Potassium solubilizing bacterial strains OPVS01, OPVS02, OPVS03, OPVS04, OPVS05, OPVS06, OPVS07, OPVS08, OPVS09, OPVS10, OPVS11, and OPVS12 (containing $\sim 10^8$ cell/mL) was used for seed treatment. The slurry of the isolate was made and then the seeds were dipped to slurry, mixed and allowed to soak for 30 min. Control seeds were not treated with cultivar. After that seed of maize were used for *in-vivo* experiment. Initial seed properties of maize cv MHM-2 seeds is given in (Table 3.5).

Table 3.4: Initial soil properties of pot experimental soil (n=3)

Soil properties	Values (mean $\pm$ SE)
Bulk Density (Mg/m ³)	1.39 $\pm$ 0.02
Particle density (Mg/m ³)	2.56 $\pm$ 0.00
Water holding capacity (%)	40.27 $\pm$ 0.51
pHw(1:2.5)	7.40 $\pm$ 0.07
EC (dS/m)	0.44 $\pm$ 0.17
Organic carbon (g/kg)	4.90 $\pm$ 0.81
Available N (mg/kg)	97.80 $\pm$ 3.57
Available P (mg/kg)	7.54 $\pm$ 1.75
Available K (mg/kg)	83.50 $\pm$ 3.84

Table 3.5: Properties of maize (cultivar MHM-2) seed used in pot experiment (n=3)

Seed properties	Values (mean $\pm$ SE)
N content (%)	1.07 $\pm$ 0.10
P content (%)	0.37 $\pm$ 0.04
K content (%)	0.39 $\pm$ 0.01
Test weight (g)	210.04 $\pm$ 0.04
Colour	Yellow

3.10.6 Experimental details

The experiment was undertaken in the nethouse of the Institute of Agricultural Sciences, Banaras Hindu University, during kharif season of the year 2013. Bioprimered seed of maize with KSB were sown in pots adopting standard procedure. Three levels of potassium (i.e. 50%, 75% and 100% recommended dose of K) and recommended dose of nitrogen and phosphorous were thoroughly mixed with soil. Experimental pots were maintained as per the treatment under the three replications of maize crop (Table 3.6).

3.10.7 Seed germination

Maize seed was treated with K-solubilizing bacterial isolates separately. Germination percentage was recorded using germination paper at ambient condition.

3.11 Plant growth study

3.11.1 Height of plants

Height of plant was measured from the surface of soil to tip of the plant with the help of a meter scale at 60 days after sowing.

3.11.2 Number of leaf/plants and stem girth

Number of leaf/plant and stem girth were recorded at 60 DAS of randomly selected plant of each replication.

3.11.4 Estimation of chlorophyll content

The chlorophyll content was estimated at 60 days after sowing of the maize crop using SPAD meter . The determination of chlorophyll contents in leaves with chemical solvents is laborious and requires lengthy procedures. In addition, field measurement of photosynthetic rate requires specialized equipment. SPAD meter which was originally developed in Japan for nitrogen management (Wood *et al.*, 1993) is now commonly used for rapid and non-destructive estimation of foliar chlorophyll concentrations. The device quantifies the relative amount of chlorophyll present in leaf by measuring the leaf transmittance in two wave bands (400-500 nm and 600-700 nm) and reports the readings in arbitrary unit.

3.11.3 Dry weight of plants

After harvesting of the crop, plant samples were kept in paper bags and dried in hot air oven at $60 \pm 2^{\circ}\text{C}$ till the constant weight.

3.11.5 Yield attributes

Yield parameters such as cob length (cm), grain/cob, test weight (g) and grain yield/plant were recorded at harvest of the maize crops

3.12 Analysis of experimental soil

Soil used in pot experiment was air dried, ground and sieved through a 2 mm sieve and analysed the soil following the standard procedure (Table 3.7).

3.12.2 Soil pH

A soil water suspension was prepared in the ratio of 1: 2.5 (10 g soil and 25 ml of distilled water) and pH was recorded with the help of a glass electrode pH meter (Jackson, 1973).

Table 3.6: Treatment details using during a nethouse experiment with maize crop¹

Notations used	KSB strain derails	Mineral fertilization (kg/ha)				
		Nitrogen	Phosphorus	100% RDK	75% RDK	50% RDK
T ₁	Uninoculated (Control)	120	60	60	45	30
T ₂	<i>A. tumefaciens</i> strain OPVS01	120	60	60	45	30
T ₃	<i>A. tumefaciens</i> strain OPVS02	120	60	60	45	30
T ₄	<i>R. pusense</i> strain OPVS03	120	60	60	45	30
T ₅	<i>R. rosettiformans</i> strain OPVS04	120	60	60	45	30
T ₆	<i>F. anhuiense</i> strain OPVS05	120	60	60	45	30
T ₇	<i>R. pusense</i> strain OPVS06	120	60	60	45	30
T ₈	<i>A. tumefaciens</i> strain OPVS07	120	60	60	45	30
T ₉	<i>F. anhuiense</i> strain OPVS08	120	60	60	45	30
T ₁₀	<i>A. tumefaciens</i> strain OPVS09	120	60	60	45	30
T ₁₁	<i>A. tumefaciens</i> strain OPVS10	120	60	60	45	30
T ₁₂	<i>A. tumefaciens</i> strain OPVS11	120	60	60	45	30
T ₁₃	<i>A. tumefaciens</i> strain OPVS12	120	60	60	45	30

¹ Recommended dose of fertilizer (RDF) 120, 60, 60 kg/ha.

3.12.3 Electrical conductivity (EC)

The same soil-water suspension, which was used for determination of pH was used to determine the electrical conductivity of soil. Soil suspension was allowed to settle till supernatant become clear. EC was measured with the help of EC meter and expressed as dS/m (Jackson, 1973).

3.12.4 Bulk density and particle density

The bulk density and particle density were determined using pycnometer bottle in laboratory in distributed soil sample (Kanwar and Chopra, 1988).

3.12.5 Organic Carbon (Walkley and Black, 1934)

One g of soil was taken in a 500 mL conical flask. 10 mL of 1N $K_2Cr_2O_7$ solution was added and mixed. 20 mL of concentrate H_2SO_4 was added, swirled the flask 2-3 times and allow kept for 30 minutes on an asbestos sheet for the reaction. The suspension was diluted with 200 mL distilled water and added a pinch of NaF and titrated against the solution with 0.5N Ferrous Ammonium Sulphate (FAS), till colour changed from deep violet to green. A blank titration was also carried out.

Table 3.7: Methods of analysis of some of the physico-chemical properties of soil

Soil Property	Method adopted	References
pHw (1:2:5)	Glass electrode pH meter	Jackson (1973)
ECw (1:2:5)	Conductivity meter	Jackson (1973)
Organic C (g/kg)	Wet digestion	Walkley and Black (1934)
Water holding capacity (%)	Gravimetric method	Kanwar and Chopra (1988)
Bulk Density ($Mg\ m^{-3}$)	Pycnometer method	Kanwar and Chopra (1988)
Particle density($Mg\ m^{-3}$)	Pycnometer method	Kanwa and Chopra (1988)
Available N (mg/kg)	Alkaline $KMnO_4$ method	Subbiah and Assija (1956)
Available P (mg/kg)	Olsen ascorbic acid	Olsen <i>et al.</i> (1965)
Available K (mg/kg)	N NH_4OAC extract	Hanway and Heidel (1952)

Calculation: % organic carbon in soil = $\frac{(B - T) \times 0.003 \times 100}{2 \times W}$

Where,

B = Volume of 0.5 N FAS Solution used for blank titration

T = Volume of 0.5 N FAS Solution used for sample titration

W = Weight of soil taken (1 g soil sample)

3.12.6 Available Nitrogen

Available Nitrogen content in soil was determined using Kjeltex Semi-Auto Nitrogen Analyzer by alkaline Potassium permanganate method as proposed by Subbiah and Asija (1956).

Five g of soil sample was transferred in a distillation tube. The sample was moistened with 5 mL distilled water, washed down the soil adhering to the neck of flask. Twenty five mL 0.32% KMnO₄ was added to it and the distillation tube was set to the instrument. In a 250 mL conical flask, 20 mL of 2 % boric acid mixed indicator was taken and placed under the receiver tube. Tap water was run continuously in the condenser. Twenty five mL of 2.5% NaOH was sucked and added to the distillation tube. Then it was put on distillation for 9 min. Nitrogen released in the form of ammonia was trapped in the boric acid, which develops green colour. The flask containing the distillate was removed. The distillate was then titrated against 0.02 N H₂SO₄ until pink colour developed.

Calculation:

$$\text{Mineralizable N (kg/ha)} = \frac{(S-V) \times 0.02 \times 14 \times 10^6 \times 2.24}{1000 \times 5}$$

S = Sample titration reading; V = Blank titration reading

3.12.7 Available P (Olsen *et al.*, 1965)

In 100 ml conical flask, 2.5 g of soil was shaken with 50 ml of 0.5 M NaHCO₃ (pH 8.5) solution and 0.5 ml of 5 N H₂SO₄ was added to it and shaken till CO₂

evolution disappeared. By the adding of 4 mL of ascorbic acid solution was used and the volume was made up using distilled water. After 10 minutes, the intensity of blue colour was recorded using spectrophotometer at 760 nm wavelength using red filter. Blank reading was also taken in the same manner without soil.

3.12.8 Available K (Jackson, 1973)

5 g soil was extracted with 25 ml of 1 N ammonium acetate (pH 7.0) by shaking for 30 minutes. The suspension was then filtered and K was determined using flame photometer.

3.12.9 Estimation of NPK in plant and grain sample

Harvested plant samples were washed in detergent solution (0.2% liquid), 0.1 N HCl solution and deionised water in sequence and dried at $70 \pm 2^\circ\text{C}$ till the constant weight. Straw and grain samples were digested in a di-acid mixture ($\text{HNO}_3:\text{HClO}_4::3:1$ v/v), and analyzed for P and K (Hanway and Heidel, 1952) by flame photometer, however, N was determined in samples digested in H_2SO_4 .

3.13 Statistical analyses

Experimental data were analysed by using analysis of variance (ANOVA) for a Factorial Complete Randomized Design (FCRD), assessed by Duncan's multiple range tests (Duncan, 1955) with a probability, the treatment mean was compared at $P < 0.05$ by using SPSS 10.0 and SAS 9.3 (SAS Institute, Cary, North Carolina, USA). For principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were used to display the correlation between the various parameters and their relationship with the different treatments.

RESULTS AND DISCUSSION

The present investigation entitled **“Isolation, Characterization and Screening of Potassium-Solubilizing Bacteria from Soils of Varanasi”** was conducted during the year 2012-2013 and a pot experiment with the maize crop under net house conditions at the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. In this chapter an attempt has been made to ascertain the degree of variations exhibited by isolation, screening, characterization of potassium solubilizing bacteria (KSB) and their effects on growth, yield along with nutrient availability (NPK) in rhizospheric soils and its uptake by maize crop influenced by various KSB strains with different levels of potassic (K) fertilization.

The data recorded during the course of the investigation were analyzed statistically for drawing the valid conclusions. To support the findings and possible causes, relevant references have been cited as per the need to justify the effect of treatment on different parameters. Differential responses exhibited by varying experimental variables on different components are described in this chapter. The whole result and discussion chapter has been described in subheads as under:

- 4.1 Collection of rhizospheric soils
- 4.2 Potassium solubilizing capacity of KSB isolates
- 4.3 Screening on the basis of K-solubilizing capacity of KSB
- 4.4 Characteristic of potassium solubilizing bacteria (KSB)
 - 4.4.1 Morphological characteristic
 - 4.4.2 Zone of solubilization
 - 4.4.3 Biochemical characteristics
 - 4.4.4 IMViC test

- 4.5 Plant growth promoting (PGP) activities of KSB strains *in-vitro*
- 4.6 Production of indole-acetic acid (IAA) by KSB strains *in-vitro*
- 4.7 Phylogeny analysis of potassium solubilization bacteria
- 4.8 Effect of KSB on pH and K release dynamics with waste muscovite and biotite
 - 4.8.1 Effect of KSB on pH dynamics with waste muscovite (WM)
 - 4.8.2 Effect of KSB on pH dynamics with waste biotite (WB)
 - 4.8.3 Effect of KSB on K-solubilization dynamics with waste muscovite (WM)
 - 4.8.4 Effect of KSB on K-solubilization dynamics with waste biotite (WB)
- 4.9 Physiological characterization of potassium solubilizing bacteria
 - 4.9.1 Temperature optimization
 - 4.9.2 pH optimization
 - 4.9.3 Salt optimization
- 4.10 Multivariate analysis
 - 4.10.1 Principal component analysis (PCA) for waste muscovite
 - 4.10.2 Principal component analysis (PCA) for waste biotite
- 4.11 Effect of KSB strains on the seed germination test
 - 4.11.1 Seed germination
 - 4.11.2 Shoot and root length
- 4.12 Effect of various KSB strains and levels of K fertilization on growth attributes of maize under net house condition
 - 4.12.1 Plant height
 - 4.12.2 Stem girth
 - 4.12.3 Chlorophyll content
 - 4.12.4 Number of leaves per plant
 - 4.12.5 Cob length of maize
- 4.13 Effect of various KSB strains and levels of K fertilization on yield attributes of maize under net house condition
 - 4.13.1 Grains per cob
 - 4.13.2 100-grain weight
 - 4.13.3 Stover yield
 - 4.13.4 Grain yield

- 4.14 Effect of various KSB strains and levels of K fertilization on maize rhizospheric nutrient availability (NPK) under net house condition
 - 4.14.1 Available nitrogen
 - 4.14.2 Available phosphorus
 - 4.14.3 Available potassium
- 4.15 Effect of various KSB strains and levels of K fertilization on nutrient (NPK) uptake (grain, stover and total) by maize crop
 - 4.15.1 Nitrogen uptake by grain
 - 4.15.2 Phosphorus uptake by grain
 - 4.15.3 Potassium uptake by grain
 - 4.15.4 Nitrogen uptake by stover
 - 4.15.5 Phosphorus uptake by stover
 - 4.15.6 Potassium uptake by stover
 - 4.15.7 Total nitrogen uptake by crop
 - 4.15.8 Total phosphorus uptake by crop
 - 4.15.9 Total potassium uptake by crop
- 4.16 PCA for growth, yield, nutrient uptake and nutrient availability in rhizospheric soil of maize crop

4.1 Collection of rhizospheric soils

The rhizospheric soil samples were taken from the root attached soils of crops/plants. Some top-surface soil was removed before the collection of rhizospheric soil samples. The surface soil was digged to ~ 10 cm soil depth, where the roots of crops were concentrated. From ~ 0 to 2.5 mm away from the root surface, a zone of soil is located that is significantly influenced by living roots and is referred to as the rhizosphere. The rhizosphere soil and roots were separated from the bulk of the soil by hand. The total 94 (45 with root-plant system) rhizospheric soil samples belonging to the dominated crop of the area such as maize (*Zea mays*), banana (*Musa paradisiaca*), tobacco (*Nicotiana tabacum*), sugarcane (*Saccharum officinarum*), pigeon pea (*Cajanus cajan*), and potato (*Solanum tuberosum*) were taken randomly in every block of Varanasi (for detail see in the section of methods and material Table

3.1). From the Arazline block 11 rhizospheric soil samples from *Z. mays*, *C. cajan*, *N. tabacum*, *S. officinarum* rhizospheric crops, from Bada Ganv 10 rhizospheric soil samples from *Z. mays*, *S. tuberosum*, *C. cajan*, *M. paradisiaca*, *S. officinarum*, from Chairaigaon block 12 rhizospheric soil samples such as from *Z. mays*, *N. tabacum*, *S. officinarum*, from Cholapur 10 rhizospheric soil samples from *Z. mays*, *S. officinarum*, *M. paradisiaca*, *C. cajan*, from harhua a total 11 rhizospheric soil samples were collected from *Z. mays*, *M. paradisiaca*, *C. cajan*, *S. tuberosum*, *S. officinarum*, *N. tabacum*.

From Khasi Vidyapeeth block a total 13 rhizospheric soil samples were collected from *Zea mays*, *Solanum tuberosum*, *Cajanus cajan* rhizospheric crops, from Pindrablok of Varanasi district a total 14 rhizospheric soil samples were collected from *Zea mays*, *Musa paradisiacal*, *Solanum tuberosum*, *Nicotiana tabacum*, *Saccharum officinarum*, and from Sewapuri block a total 13 rhizospheric soil samples were collected from *Zea mays*, *Cajanus cajan*, *Saccharum officinarum*, *Nicotiana tabacum*. From the all eight block of Varanasi district a total 94 rhizospheric soil samples were collected from six dominated crops with maize was common crops selected from all blocks (Table 4.1). Approximate twenty gram (~ 20 g) of rhizosphere soil samples were collected around each crop plants.

The rhizospheric soil samples were collected from different crop fields covering a total area of 2,535 km² from June to August 2012. All crop fields were located at Indo-Gangetic Plains of Ganges river in the holy city Varanasi (El 80.71 m [264.80 ft]; Lt25.3333° N; Lg 83.0000° E), in the state of Uttar Pradesh (Fig. 4.1). The rhizosphere soil samples were collected in rainy season (June to August), which is the peak growing season for the crops mentioned above. All the rhizospheric soil samples were sealed in sterilized ziplock bags, stored in an ice chest, and used within 10-14 h of collection.

4.2 Isolation and purification of potassium solubilizing bacteria

Modified solid Aleksandrov medium (MAM_s) supplemented with waste mica (muscovite) powder as a sole source of K, was used to screen K-solubilizing bacteria

from the rhizospheric soil. The serially diluted (in sterile normal saline, 0.87%) soil samples were plated on MAM_s containing (per liter) 5 g glucose, 0.005 g MgSO₄.7H₂O, 0.1 g Fe Cl₃, 2.0g CaCO₃, 3.0 waste mica (muscovite and biotite) as a potassium mineral (2.0 g used in original media), 2.0 g calcium phosphate and 20 g agar-agar (Hu *et al.*, 2006). The plates were then incubated at 28 ± 2°C. After 3 days, colonies showing formation of clear zone around them were considered to be KSB or KSB and selected for further studies using Aleksandrov broth medium (Sugumaran and Janartham, 2007).

A total of 30 different types colonies were able to grow on MAMs which were from rhizosphere of different crops from Indo Gangetic Plain (IGP) of India. Among these isolated colonies, only 21 were found to make a zone of clearance indicating K solubilization on MAM_s. Among the 21 KSB strain on the basis of K-solubilization only twelve KSB strain was selected for future study. Out of these 12 bacterialisolates, 4 belonged to rhizosphere of maize, 2 each of banana, sugarcane and potatoand, 1 each to pigeon pea and tobacco rhizosphere (Table 4.1). The KSB strain or isolates can be obtained from the crop rhizosphere easily as also done by Altomare *et al.* (1999) and replicated in our studies. All bacterial strains isolated in our study produced slime; however, magnitude of production varied from low to high depending upon the capacity of individual KSB strains. All purified KSB strains were stored at 4°C in slant for furthered study.

Screened isolates were gram-stained for presumptive identification and pure colonies were transferred to sterile slants on Nutrient Agar medium (Hi-Media). Further, in another set of experiment, a zone of solubilization of purified KSB isolates was measured using a scale after 7 days of plating on MAMs. The KSB strains were purified by repeated sub-culturing on their respective medium by streaking method. Individual colonies from streaked plates were picked up and checked for their purity under microscope. The pure colony was transferred in their respective slants and incubated and stored at 4°C in the refrigerator for further study.

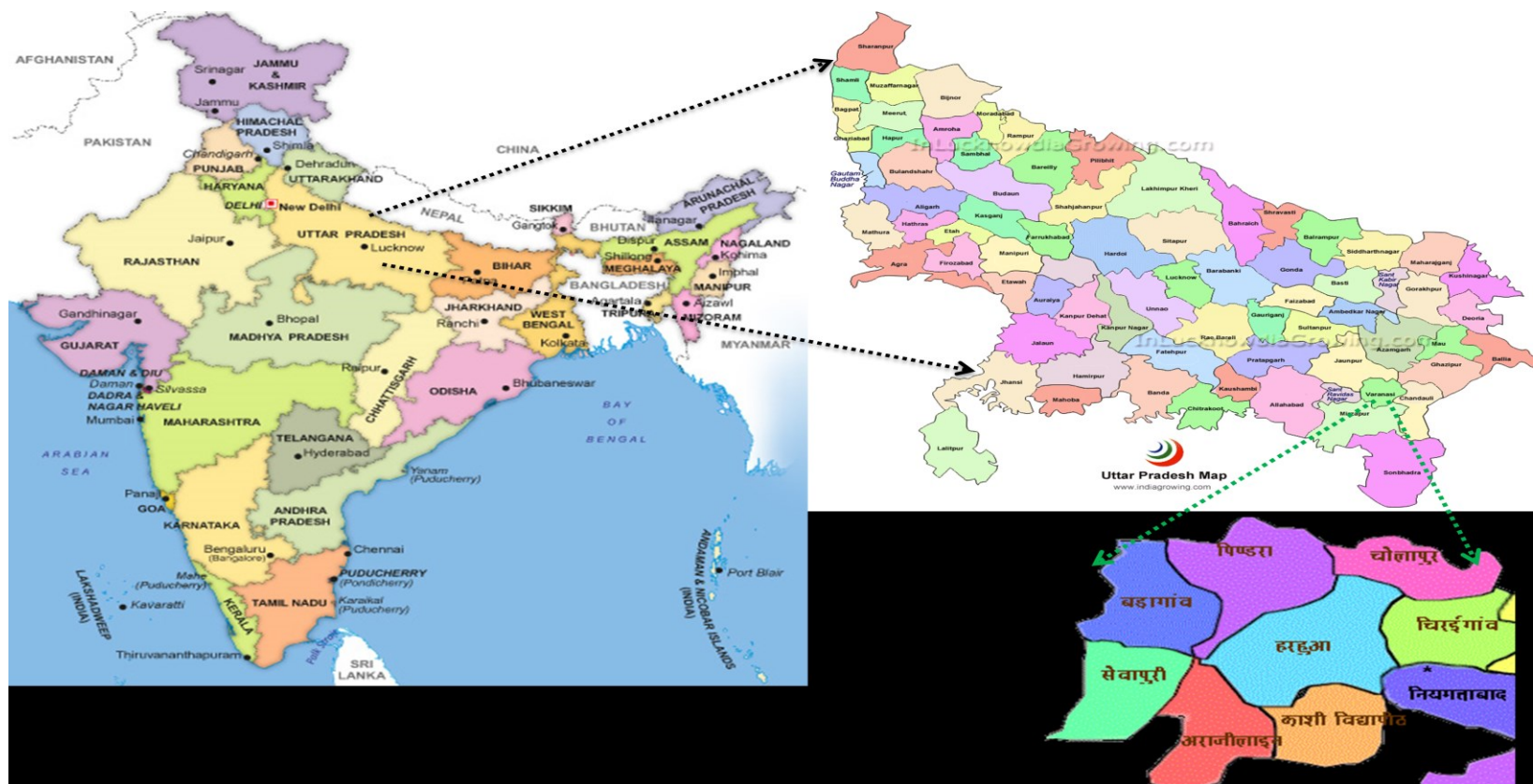


Fig. 4.1: Sampling site for collected rhizospheric soil samples (from June to August, 2012) from the Varanasi district in the state of Uttar Pradesh, North Central Part of India.

Table 4.1: Potassium solubilizing capacity of KSB isolates after 10 days of incubation under *in-vitro*

Isolate no.	Potassium solubilization (µg/mL)	Isolate no.	Potassium solubilization (µg/mL)	Isolate no.	Potassium solubilization (µg/mL)	Isolate no.	Potassium solubilization (µg/mL)
1	8.47 ± 0.37	33	19.39 ± 0.54	65	11.89 ± 0.83	97	15.64 ± 1.92
2	11.16 ± 0.59	34	10.11 ± 0.22	66	10.83 ± 0.85	98	12.45 ± 0.42
3	10.23 ± 0.58	35	20.18 ± 1.05	67	21.74 ± 1.54	99	10.44 ± 0.53
4	8.43 ± 0.61	36	20.63 ± 0.88	68	8.34 ± 0.60	100	10.10 ± 0.50
5	8.10 ± 0.35	37	8.12 ± 0.35	69	15.89 ± 2.97	101	12.19 ± 1.59
6	20.64 ± 0.84	38	21.70 ± 0.68	70	12.64 ± 0.36	102	9.35 ± 0.46
7	20.24 ± 1.02	39	8.55 ± 0.49	71	9.54 ± 0.34	103	13.03 ± 0.78
8	12.56 ± 0.38	40	8.60 ± 0.47	72	10.97 ± 1.03	104	11.99 ± 2.08
9	11.49 ± 0.39	41	21.55 ± 0.35	73	19.31 ± 0.48	105	12.42 ± 1.45
10	10.24 ± 0.58	42	9.41 ± 0.52	74	19.93 ± 0.54	106	9.32 ± 0.58
11	20.17 ± 1.10	43	21.45 ± 1.14	75	13.46 ± 0.38	107	10.13 ± 2.07
12	8.03 ± 0.45	44	10.24 ± 0.71	76	8.54 ± 0.63	108	20.72 ± 0.71
13	9.85 ± 0.23	45	10.64 ± 1.15	77	11.37 ± 0.48	109	9.06 ± 1.13
14	16.37 ± 1.26	46	7.90 ± 0.30	78	21.64 ± 1.15	110	19.70 ± 0.48
15	13.08 ± 1.65	47	10.78 ± 1.01	79	8.80 ± 0.33	111	20.33 ± 0.90
16	11.80 ± 0.73	48	11.55 ± 1.14	80	9.57 ± 0.49	112	21.06 ± 1.10
17	21.41 ± 0.60	49	8.50 ± 0.29	81	9.71 ± 0.85	113	20.30 ± 0.61
18	10.11 ± 1.11	50	8.07 ± 0.17	82	11.87 ± 0.70	114	10.23 ± 0.61
19	10.20 ± 0.62	51	7.16 ± 0.74	83	9.29 ± 0.64	115	10.85 ± 0.81
20	9.87 ± 1.70	52	11.54 ± 0.72	84	15.27 ± 0.73	116	12.09 ± 0.29
21	18.16 ± 0.86	53	9.84 ± 0.71	85	13.88 ± 0.92	117	7.75 ± 0.91
22	18.75 ± 0.23	54	9.31 ± 0.58	86	21.94 ± 0.86	118	11.12 ± 1.81
23	19.39 ± 0.50	55	21.68 ± 1.11	87	8.80 ± 0.62	119	12.53 ± 1.75
24	8.38 ± 0.67	56	9.52 ± 0.54	88	11.31 ± 0.64	120	14.25 ± 0.58
25	9.50 ± 0.44	57	21.31 ± 0.94	89	8.23 ± 0.55	121	11.83 ± 0.50
26	13.94 ± 1.41	58	10.53 ± 0.52	90	9.40 ± 0.43	122	10.27 ± 0.67
27	10.06 ± 0.19	59	8.38 ± 0.96	91	9.21 ± 1.04	123	10.08 ± 1.61
28	9.07 ± 0.28	60	11.80 ± 0.76	92	8.55 ± 0.49	124	9.69 ± 2.19
29	20.30 ± 0.41	61	23.13 ± 1.56	93	10.57 ± 0.68	125	11.24 ± 0.68
30	10.24 ± 0.70	62	8.98 ± 0.83	94	8.36 ± 0.65	126	12.25 ± 1.40
31	9.54 ± 0.60	63	11.75 ± 1.73	95	18.97 ± 1.48	127	12.57 ± 0.34
32	10.45 ± 0.42	64	12.03 ± 1.82	96	8.89 ± 0.69		

4.3 Screening on the basis of K-solubilizing capacity of the KSB for onward study

Waste muscovite (WM) powder was added to the modified Aleksandrov broth (MAB) as the sole source of K to test the K-solubilization ability of the all purified KSB strains. Conacial flask containing 50 mL sterilizes MAB were sterilized by autoclaving at 0.1 MPa for 20 min. The cooled flasks were inoculated with 1 mL KSB culture raised to a population of 10^8 CFU/mL by overnight shaking (150 RPM, $28 \pm 1^\circ\text{C}$). A total 127 KSB stains were used for the screening on the basis of K-solubilization. The K-solubilization varied among the KSB strains with its range from 7.16-to-23.13 $\mu\text{g/mL}$. On the basis of the K-solubilization top twelve KSB strains were screened out for further study (Table 4.2).

Table 4.2: K-solublizing bacterial isolates selected for pot and lab experiments¹

Primary numbering	Decoding	K-solubilization($\mu\text{g/mL}$)
17	<i>A. tumefaciens</i> strain OPVS01	21.41
38	<i>A. tumefaciens</i> strain OPVS02	21.70
41	<i>R. pusense</i> strain OPVS03	21.55
43	<i>R. rosettiformans</i> strain OPVS04	21.45
55	<i>F. anhuiense</i> strain OPVS05	21.68
57	<i>R. pusense</i> strain OPVS06	21.31
61	<i>A. tumefaciens</i> strain OPVS07	23.13
67	<i>F. anhuiense</i> strain OPVS08	21.74
78	<i>A. tumefaciens</i> strain OPVS09	21.64
86	<i>A. tumefaciens</i> strain OPVS10	21.94
108	<i>A. tumefaciens</i> strain OPVS11	20.72
112	<i>A. tumefaciens</i> strain OPVS12	21.06

Twelve efficient isolates were selaectedfor further study (Table 4.2). KSB strains were recorded as *A. tumefaciens* strain OPVS01 (21.41 $\mu\text{g/mL}$), *A. tumefaciens*strain OPVS02 (21.70 $\mu\text{g/mL}$), *R. pusense* strain OPVS03 (21.55 $\mu\text{g/mL}$),

¹ On the basis of K-solubilizing capacity, 12 efficient KSB strains were selected for further study

R. rosettiformans strain OPVS04 (21.45 µg/mL), *F. anhuiense* strain OPVS05 (21.68 µg/mL), *R. pusense* strain OPVS06 (21.31 µg/mL), *A. tumefaciens* strain OPVS07 (23.13 µg/mL), *F. anhuiense* strain OPVS08 (21.74 µg/mL), *A. tumefaciens* strain OPVS09 (21.64 µg/mL), *A. tumefaciens* strain OPVS10 (21.94 µg/mL), *A. tumefaciens* strain OPVS11 (20.72 µg/mL) and *A. tumefaciens* strain OPVS12 (21.06 µg/mL).

4.4 Characteristic of potassium solubilizing bacteria

4.4.1 Morphological characterization

Among the twelve KSB stains, nine KSB strain showed gram positive reactions and the rest of the three showed gram negative reaction. The KSB strain namely *A. tumefaciens* strain OPVS01, *A. tumefaciens* strain OPVS02, *R. pusense* strain OPVS03, *R. rosettiformans* strain OPVS04, *F. anhuiense* strain OPVS05, *R. pusense* strain OPVS06, *A. tumefaciens* strain OPVS07, *A. tumefaciens* strain OPVS09 and *A. tumefaciens* strain OPVS10 showed gram positive reaction. However, *F. anhuiense* strain OPVS08, *A. tumefaciens* strain OPVS11 and *A. tumefaciens* strain OPVS12 showed gram negative reaction (Table 4.3).

Morphology KSB colony was revealed white, creamy and whitish with smooth and rough surface. OPVS-2, 9 and 11 were noted for higher slime producers while OPVS-1, 6 and 10 had a medium capacity to produce slime. Out of 12 KSB strains, 6 strains showed entire smooth margin. OPVS-3, 5, 6, 7, and 10 showed slightly elevated colonies while colonies of OPVS-1, 2, 4, 8, 9, 11 and 12 were highly raised. On MAMs, a colony formed by most of the isolates appeared to be translucent except colonies of OPVS-2, 3, and 4 which appeared to be opaque. Semi-solid agar medium was designed to demonstrate motility by diffusion. This method is to determine if the bacteria motile by means of flagellum. Non-motile bacteria do not produce flagellum. Out of 12 KSB strains, eight KSB strains such as OPVS-1, 2, 3, 7, 9, 10, 11 and OPVS-12 showed the motility (Table 4.4). However, only four KSB strains such as OPVS-4, 5, 6 and 8 results were no-motile. The KSB strains exhibited motility which is another interesting feature since it promotes colonization of roots.

Table 4.3: Colony characteristics of KSB isolated from different crops on Modified Aleksandrov Medium (MAMs)²

Isolates	Colour	Margin	Gram reaction	Colony elevation		Optical Density		Slime production
				Slightly raised	Highly raised	Translucent	Opaque	
OPVS-1	White	Smooth	+	–	+	+	–	Medium
OPVS-2	Whitish	Rough	+	–	+	–	+	High
OPVS-3	White	Smooth	+	+	–	–	+	Low
OPVS-4	Creamy	Rough	+	–	+	–	+	Low
OPVS-5	White	Rough	+	+	–	+	–	High
OPVS-6	Creamy	Smooth	+	+	–	+	–	Medium
OPVS-7	Creamy	Smooth	+	+	–	+	–	Low
OPVS-8	White	Rough	–	–	+	+	–	Low
OPVS-9	White	Smooth	+	–	+	+	–	High
OPVS-10	Creamy	Smooth	+	+	–	+	–	Medium
OPVS-11	White	Rough	–	–	+	+	–	High
OPVS-12	Creamy	Smooth	–	–	+	–	+	Low

²+ Presence; – Absence

Diverse groups of microorganisms, including *Pseudomonas*, *Serratia*, *Acinetobacter* spp. employ a variety of solubilization reactions, such as acidification, chelation, exchange reactions, and production of gluconic acid to release soluble from insoluble P (Kumar *et al.*, 2015; Maurya *et al.*, 2015).

4.4.2 Zone of solubilization

A potassium source waste mica (waste muscovite) powder agar plate was used in the quantitative measurement of the K solubilization zone ability of the KSB. All the bacteria were found to be capable for solubilizing K and the solubilization zone ranged from 1.29 to 2.34 cm in diameter. Strain OPVS 11 showed most pronounced ability to solubilize K (2.34 cm) followed by OPVS 6 and 10 (Fig. 4.2). However, OPVS 1 solubilized the least amount of K as observed by weak zone of 1.29 cm as compared to other strains used in this study (Fig. 4.3). Data recorded on the zone of solubilization revealed that OPVS-11 which was developed from rhizosphere of *Zea mays* formed highest zone of solubilization as compared to other KSB strains.

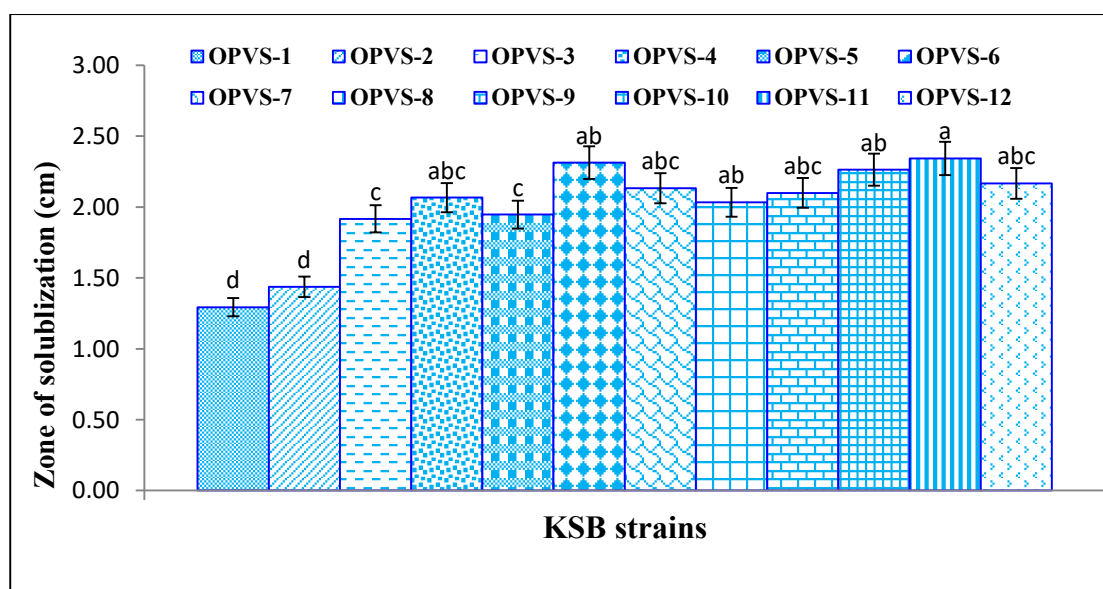


Fig. 4.2: Zone of solubilization of different KSB strain at 7 DAI on MAMs (waste muscovite)

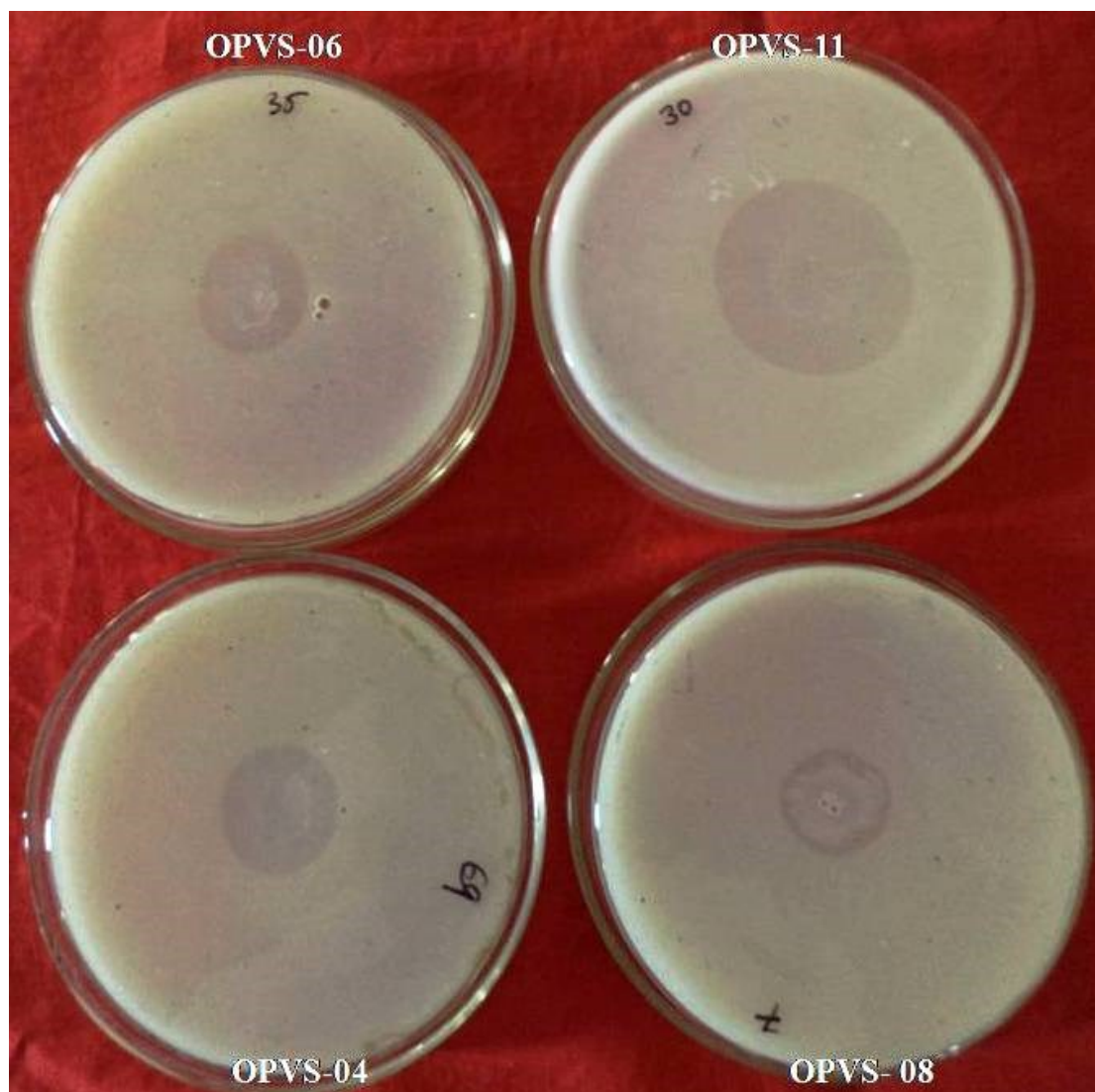


Fig. 4.3: Solubilization zone on MAMs at 7 days after incubation, *Rhizobium pusense* strain OPVS06, *Agrobacterium tumefaciens* strain OPVS11; *R. rosettiformans* strain OPVS04; *Flavobacterium anhuiense* strain OPVS08.

The solubilization zones by different strains were found to vary from 1.29 to 2.34 cm on MAMs. Interestingly, strains from cereals (maize) caused much greater zone of solubilization when compared with the strains isolated from pulses (pigeon pea). Several studies have shown that the soil contains a variety of KSB as also found out in our study (Friedrich *et al.*, 2004; Meena *et al.*, 2013). These rhizospheric microorganisms mineralize or decompose silicate minerals such as K-feldspar and waste mica (both waste muscovite and biotite). They solubilize fixed form of K in the

soil into plant available K that can be directly absorbed by plants, leading to enhanced plant growth promotion and development, although they might also secrete other active plant growth promoting substances (Meena *et al.*, 2014a). Recently, the use of K-solubilizers as biological fertilizers has been considered as a hot spot while studying agriculture and environmental conservation (Meena *et al.*, 2014b).

4.4.3 Biochemical characterization

General biochemical characterization of KSB strains presented in table 4.4. Out of 12 KSB strains 50% KSB strain showed positive and 50% isolates showed negative to OPVS-1, 3, 6, 8, 10 and 12 showed positive starch hydrolysis, however, another six KSB strain, OPVS-2, 4, 5, 7, 9 and 11 showed negative starch hydrolysis.

All twelve KSB strains produce acid, but its varied among the KSB strain as a low, medium and high. Among the 12 KSB strains, 5 KSB (OPVS-1, 2, 3, 7 and 9) strains produce higher acid as compared to the rest of the KSB strains. Only two KSB strains OPVS-4 and 8 showed the lower amount of acid production. While OPVS-5, 6, 8, 11 and 12 KSB strains showed medium production of acid. In case of urease test among the 12 KSB strains each six KSB strains showed a positive and negative reaction. OPVS-1, 4, 6, 8, 10, and 12 showed presences positive test for urease, however, OPVS-2, 3, 5, 7, 9 and 11 showed negative in urease test. In case of hydrogen sulfide (H₂S) production among the 12 KSB strains only 5 KSB strain such as produce OPVS-1, 3, 6, 8 and 12 produce H₂S gas. However, most of the KSB strain doesn't produce H₂S gas like OPVS-2, 4, 5, 7, 8, 10 and 11. Catalase test most of the KSB strain (OPVS-1, 2, 4, 5, 6, 7, 10, 11 and 12) was positive in catalase test and rest three KSB strains showed OPVS-3, 8 and 9 were negative in catalase test.

4.4.4 IMViC test

The KSB strains OPVS-1, 3, 6, 8, 10, and 12 showed the positive methyl red test (MR) and the rest of KSB strains such as OPVS-2, 4, 5, 7, 9 and 11 showed negative MR test.

Table 4.4: Biochemical characteristics of KSB isolated from different crops rhizosphere³

Isolates	Motility	Starch Hydrolysis	H ₂ S	Acid Production	Urease	Catalase
OPVS-1	+	+	+	+++	+	+
OPVS-2	+	–	–	+++	–	+
OPVS-3	+	+	+	+++		–
OPVS-4	–	–	–	+	+	+
OPVS-5	–	–	–	++	–	+
OPVS-6	–	+	+	++	+	+
OPVS-7	+	–	–	+++	–	+
OPVS-8	–	+	–	+	+	–
OPVS-9	+	–	+	++	–	–
OPVS-10	+	+	–	+++	+	+
OPVS-11	+	–	–	++	–	+
OPVS-12	+	+	+	++	+	+

³+ Presence; – Absence; + Low; ++ Medium; +++ High

Results and Discussion

However, in case of Voges-Proskauer (VP) test out of twelve KSB strains, five KSB strains OPVS-1, 3, 6, 9 and 12 showed positive reactions while OPVS-2, 4, 5, 6, 7, 8, 10 and 11 KSB strains showed negative in Voges-Proskauer (VP) test (Table 4.6). The entire KSB strain results showed positive citrate utilization test, which varied as low, medium and high. Out of 12 KSB strains 5 KSB strain showed higher amounts of citrate. However, KSB strains OPVS-4 and 8 results showed the lower value of citrate utilization.

Table 4.5: IMViC tests of different KSB isolates

Isolates	Methyl red (MR)	VogesProskauer (VP)	Citrate Utilization
OPVS-1	+	+	+++
OPVS-2	–	–	+++
OPVS-3	+	+	+++
OPVS-4	–	–	+
OPVS-5	–	–	++
OPVS-6	+	+	++
OPVS-7	–	–	+++
OPVS-8	+	–	+
OPVS-9	–	+	++
OPVS-10	+	–	+++
OPVS-11	–	–	++
OPVS-12	+	+	++

+ Presence; – Absence; + Low; ++ Medium; +++ High

H₂S is a harmful byproduct of many normal metabolic processes to prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules.

4.5 Plant growth promoting (PGP) activities of KSB strains *in-vitro*

The PGP activities of KSB strains are presented in table 4.6. Gibberellic acid, HCN, ammonia production and oxidase test were carried out for all twelve KSB

Results and Discussion

strains. Different PGP activates varied among the different KSB strains. Out of twelve KSB strains, five KSB strains, OPVS-1, 3, 6, 9 and 12 showed positive reaction for gibberellic acid production and rest of the KSB strains OPVS-2, 4, 5, 7, 8, 10 and 11 showed negative gibberellic acid production.

Table 4.6: Plant growth promoting (PGP)activities of KSB isolated from different crops rhizosphere⁴

Isolates	Gibberellic acid	HCN	Ammonium	Oxidase
OPVS-1	+	–	+++	+
OPVS-2	–	+	+++	+
OPVS-3	+	+	+++	+
OPVS-4	–	–	+	+
OPVS-5	–	+	++	+
OPVS-6	+	+	++	+
OPVS-7	–	+	+++	+
OPVS-8	–	–	+	+
OPVS-9	+	+	++	+
OPVS-10	–	–	+++	+
OPVS-11	–	+	++	+
OPVS-12	+	+	++	+

Eight KSB strains, OPVS-2, 3, 5, 6, 7, 9, 11 and 12 showed positive HCN production and the rest of the strain was negative in HCN production. However, in case of ammonia production (NH₃) it's varied among the KSB strains as a low, medium and high. Out of 12 KSB strain 5 KSB strain showed the high amount of NH₃ production and another five KSB strain showed the medium in NH₃ production and rest of two showed less production of NH₃. The production of oxidase was a common trait in all KSB strains. All the twelve KSB strain showed positive oxidase test (Table 4.7). Siddiquiet *al.* (2006) reported that HCN is a volatile, secondary

⁴+ Presence; – Absence; + Low; ++ Medium; +++ High

metabolite that suppresses the development of microorganisms and that also affects negatively the growth and development of plants (Kang *et al.*, 2014).

4.6 Production of indole-acetic acid (IAA) by KSB strains *in-vitro*

All the KSB strains were tested for the quantitative estimation of IAA in the presence of 25, 50, 75 and 100 µg/mL concentrations of tryptophan (Table 4.8) at 48 h after incubation. In the absence of tryptophan, there was no production of IAA as tryptophan is a key precursor of IAA biosynthesis. IAA concentration varied according to efficiency of KSB strains. At 25 µg/mL concentration of tryptophan significantly higher production of IAA was recorded with OPVS-4 and it was at par with OPVS-10 KSB strain. Significantly lower IAA production was recorded with OPVS-6 KSB strain were compared with control.

Results with 50 µg/mL concentrations of tryptophan showed that the significantly higher (9.90 µg/mL) IAA production was with OPVS-10 which was significantly superior to other KSB strains. However, in case of 75 µg/mL concentration of tryptophan significantly higher (13.90 µg/mL) IAA production was recorded with OPVS-8 followed by OPVS-9, 10, 11 and 12. The use of 100 µg/mL concentration of tryptophan, showed significantly higher production of IAA with OPVS-10 and 11 (21.01 and 21.25 µg/mL), respectively. Interaction effects of KSB strains and concentrations of tryptophan for IAA production were significantly influenced. The significantly higher IAA production was also observed with 100 µg/mL concentrations of tryptophan followed by 75, 50 and 25 µg/mL concentrations of tryptophan (Fig. 4.4). IAA production by bacteria are considered as an effective tool for screening by growth promoting microorganisms, as many reports suggest that IAA producing bacteria have a profound effect on plant growth (Govindarajan *et al.*, 2007; Ali and Hasnain, 2007). They promote lateral and adventitious root formation, which can facilitate high root surface area for nutrient absorption from soil (Chaihar and Lumyong, 2011; Meena *et al.*, 2014a).

Table 4.7: Indole-acetic acid production after 48 h of incubation with different KSB strains *in-vitro*

Isolates	IAA at different tryptophan ($\mu\text{g/mL}$)			
	25	50	75	100
OPVS-1	$4.97 \pm 0.23^{\text{ef}}$	$6.71 \pm 0.36^{\text{e}}$	$9.76 \pm 0.25^{\text{f}}$	$13.58 \pm 0.33^{\text{c}}$
OPVS-2	$6.12 \pm 0.31^{\text{cde}}$	$7.27 \pm 0.18^{\text{de}}$	$10.92 \pm 0.33^{\text{def}}$	$15.21 \pm 0.58^{\text{c}}$
OPVS-3	$7.10 \pm 0.31^{\text{bc}}$	$8.63 \pm 0.18^{\text{bc}}$	$11.58 \pm 0.67^{\text{cde}}$	$18.19 \pm 0.88^{\text{b}}$
OPVS-4	$8.80 \pm 0.35^{\text{a}}$	$9.36 \pm 0.34^{\text{bc}}$	$13.92 \pm 0.33^{\text{a}}$	$17.41 \pm 0.66^{\text{b}}$
OPVS-5	$6.72 \pm 0.37^{\text{cd}}$	$8.00 \pm 0.29^{\text{cd}}$	$10.48 \pm 0.37^{\text{ef}}$	$15.01 \pm 0.24^{\text{c}}$
OPVS-6	$4.41 \pm 0.64^{\text{f}}$	$7.02 \pm 0.28^{\text{e}}$	$12.25 \pm 0.58^{\text{bcd}}$	$14.34 \pm 0.59^{\text{c}}$
OPVS-7	$5.81 \pm 0.36^{\text{cdef}}$	$7.38 \pm 0.31^{\text{de}}$	$11.25 \pm 0.58^{\text{cdef}}$	$14.25 \pm 0.58^{\text{c}}$
OPVS-8	$5.36 \pm 0.69^{\text{def}}$	$7.15 \pm 0.16^{\text{de}}$	$13.90 \pm 0.33^{\text{a}}$	$17.85 \pm 0.67^{\text{b}}$
OPVS-9	$5.36 \pm 0.14^{\text{def}}$	$7.17 \pm 0.20^{\text{de}}$	$12.58 \pm 0.33^{\text{abc}}$	$18.30 \pm 0.78^{\text{b}}$
OPVS-10	$8.25 \pm 0.25^{\text{ab}}$	$9.90 \pm 0.06^{\text{a}}$	$13.25 \pm 0.58^{\text{ab}}$	$21.01 \pm 0.80^{\text{a}}$
OPVS-11	$7.30 \pm 0.71^{\text{bc}}$	$9.17 \pm 0.44^{\text{bc}}$	$12.58 \pm 0.67^{\text{abc}}$	$21.25 \pm 0.58^{\text{a}}$
OPVS-12	$5.25 \pm 0.69^{\text{def}}$	$7.00 \pm 0.06^{\text{e}}$	$13.36 \pm 0.49^{\text{ab}}$	$18.07 \pm 0.96^{\text{b}}$

It has been reported that IAA production by PGP bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza *et al.*, 2001). In spite of being good producers of IAA, these bacteria were also positive for potassium and phosphate solubilization (Jha and Kumar, 2009; Chaiarn and Lumyong, 2011). Similarly, high levels of IAA production were recorded in *Pseudomonas* by other workers (Ahmad *et al.*, 2008; Kumar *et al.*, 2014). Bent *et al.* (2001) reported that the production of indole compounds by three different strains, *Paenibacilluspolymyxa* L6, *P. polymyxa* Pw-2, and *Pseudomonas fluorescens* M20 increased in concentration with increasing concentrations of tryptophan (0-200 mg/mL) at different times. The IAA production

was detected in all KSB strains suspensions in this study and they have a beneficial association with the plants as evidenced by increase in plant dry weight and nutrient uptake (Zhang and Kong, 2014).

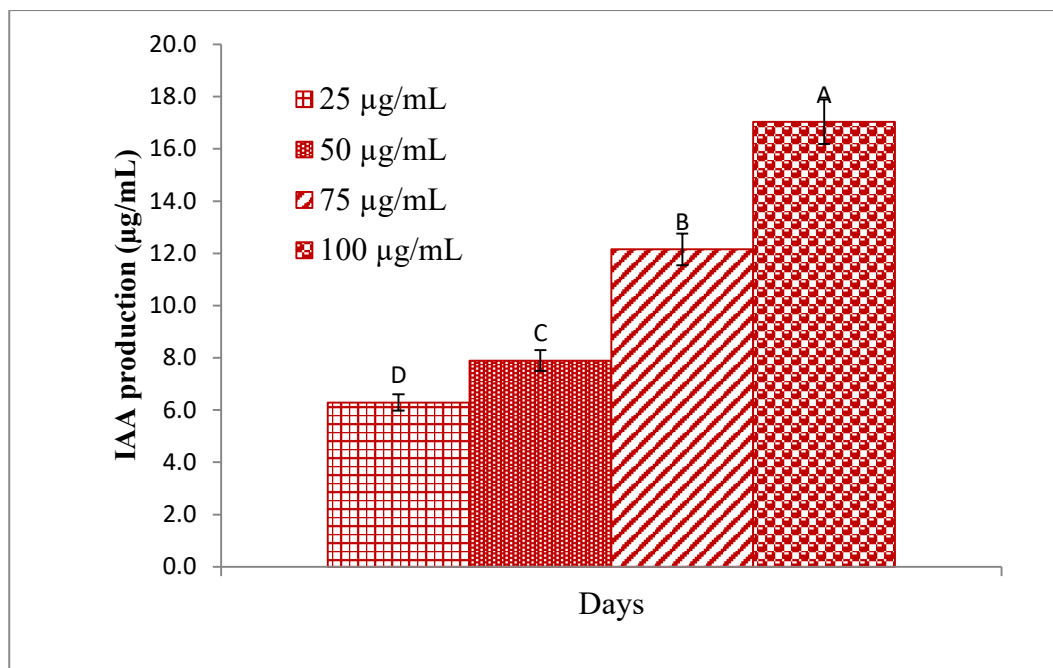


Fig. 4.4: Interaction effect of KSB on IAA production at different concentration of tryptophan after 48 hrs (2 DAI) of incubation⁵.

4.7 Phylogenic analysis of potassium solubilizing bacteria (KSB)

Twelve isolates examined in this study affirmed to 4 different species of KSB, based on 16S phylogeny, isolated from maize, banana, sugarcane, potato, pigeon pea and tobacco rhizosphere (Fig. 4.5). The 16S rDNA sequences of the 12 KSB strains were compared to those of known 16S rDNA sequences using BLAST and the results indicate that the 12 KSB strains isolated from different rhizosphere can be basically categorized into four groups (Table 4.9). Most of the KSB obtained so far by other workers have been isolated from rhizosphere of different plants. Previously KSB have been successfully isolated from rhizosphere of rice, corn, tobacco and coconut plantations corroborating our findings (Zhang and Kong, 2014; Meena *et al.*, 2015).

⁵Bars followed by a similar letter between treatments within a concentration of tryptophan are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

B. mucilaginosus, *Bacillus* and *Pseudomonas* species are the most widely studied species of KSB (Zhou *et al.*, 2006; Sugumaran and Janarthanam, 2007). In our study, seven isolates (58.33%) were found to be closely phylogenetically related to *Agrobacterium*, showing about 99-100% similarity in their 16S rDNA sequences, making *Agrobacterium* was the most dominant genus namely *Agrobacterium tumefaciens* strain (58.33%/7 out of 12), *Rhizobium pusense* strain (16.66%/2 out of 12), *Flavobacterium anhuiense* strain (16.66%/2 out of 12), and *Rhizobium rosettiformans* strain (8.33%/ 1 out of 12).

The results indicate that there is a greater diversity of the K-solubilizing bacteria waiting to be discovered and the vast benefits they bring with them is the present answer to many of the synthetic fertilizer conundrum. The 16S rDNA gene characterization confirmed the identification of KSB and prepared phylogenetic relationships among 16S rDNA (Fig. 4.5).

4.8 Effect of KSB on pH and K release dynamics with waste muscovite and biotite broth

4.8.1 Effect of KSB on pH dynamics with waste muscovite (WM) broth

Initial pH of uninoculated waste muscovite (WM) added broth was 7.6 which did not influence much due to incubation period. However, a slight change in pH values with incubation period was observed. This may be due to production of H^+ during the hydrolysis of added waste muscovite. Bin *et al.* (2010) also reported a very minute change in pH of mineral added broth with increase in incubation periods. The pH value of inoculated broth supplemented with waste muscovite decreased significantly by all KSB strains with an increase in the incubation period. All the KSB strains caused significant decrease in pH of broth as compared to uninoculated waste muscovite broth at 7, 14 and 21 DAI, (Fig. 4.6a). At all the incubation periods, strain OPVS 11 showed significantly lower pH value as compared to other KSB strains. However, OPVS-1 and 12 KSB strains showed in a very minute change in pH of the MAB as compared to rest of the KSB strains (Table 4.6).

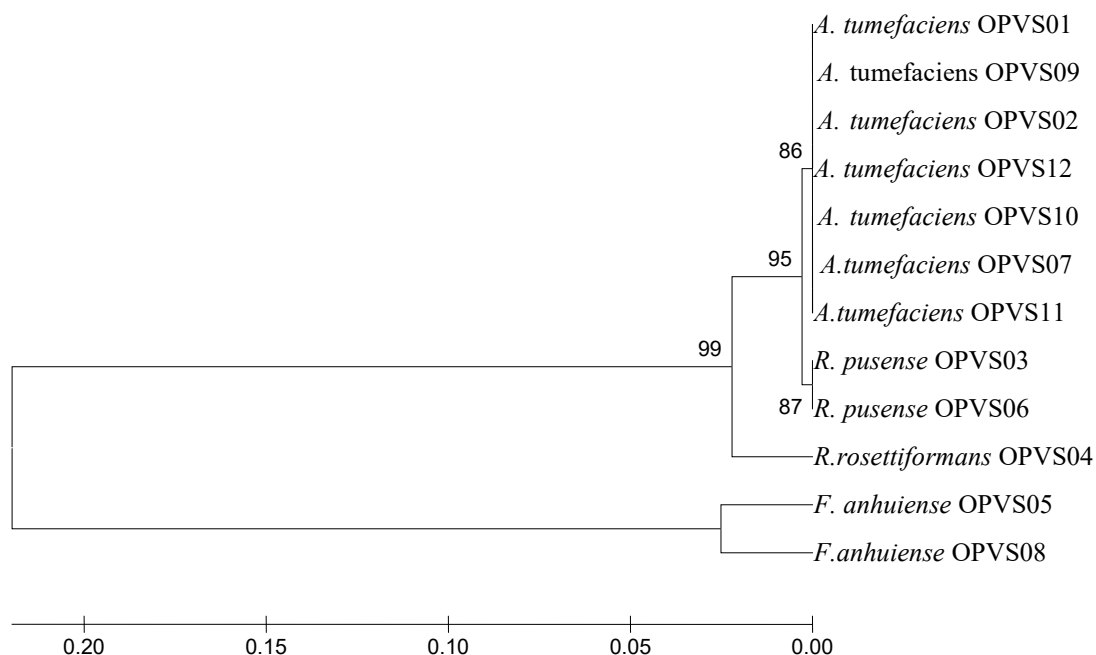


Fig. 4.5: Phylogenetic relationships among 16S rDNA sequences of KSB, Bootstrap values (> 50%) are indicated at branch points and scale bar 0.05 variation.

Interaction effects of KSB with incubation period regarding pH significantly differed with each other. The significantly lower value of pH was observed at 21 DAI which was at par with 14 DAI. But at 7 DAI differed significantly (Fig. 4.6a). The decrease in pH due to KSB strains varied with the acid production capacity of the different KSB strains (Zhang and Kong, 2014; Maurya *et al.*, 2015). Decrease in pH in the presence of the bacterial strain might be due to the release of some organic acid by microorganisms. It was reported that the bacterial intervention on silicate minerals is closely associated with the formation of mucilaginous compounds consisting of EPS as well as with the production of different low molecular organic acids (Groudeva and Groudev, 1987). It is also reported that mineral K-solubilization is due to the production of low molecular acids by soil microorganisms (Leyval and Berthelin, 1989). Protons associated with organic acid molecules decrease the pH of the solution and, therefore, induces the releasing capacity of cations such as iron, potassium and magnesium.

Table 4.8: Genetic characterization of selected potassium solubilizing bacterial (KSB) strains to gene level

Rhizosphere of crop	GeneBank accession number	Closest species with accession number	Similarity (%)	Isolates denoted
<i>Zea mays</i>	KJ410659	<i>A. tumefaciens</i> strain D254 (KJ499777)	100	<i>A. tumefaciens</i> strain OPVS01
<i>Musa paradisiaca</i>	KJ410660	<i>A. tumefaciens</i> , isolate BD18-R03 (HF584882)	100	<i>A. tumefaciens</i> strain OPVS02
<i>Solanumtuberosum</i>	KJ410661	<i>R. pusense</i> strain BN-23 (AB969785)	100	<i>R. pusense</i> strain OPVS03
<i>Cajanuscajan</i>	KJ410662	<i>R. rosettiformans</i> strain W3 (NR116445)	99	<i>R. rosettiformans</i> strain OPVS04
<i>Nicotianatabacum</i>	KJ410663	<i>F. ahuensis</i> strain THWCS2 (GQ284450)	91	<i>F. anhuiense</i> strain OPVS05
<i>Saccharumofficinarum</i>	KJ410664	<i>R. pusense</i> strain BN-23 (AB969785)	100	<i>R. pusense</i> strain OPVS06
<i>Zea mays</i>	KJ410665	<i>A. tumefaciens</i> isolate Y36 (KF730752)	100	<i>A. tumefaciens</i> strain OPVS07
<i>Zea mays</i>	KJ410666	<i>F. ahuensis</i> strain THWCSN17 (GQ284447)	94	<i>F. anhuiense</i> strain OPVS08
<i>Musa paradisiaca</i>	KJ410667	<i>A. tumefaciens</i> strain SWFU-W99 (KF773134)	100	<i>A. tumefaciens</i> strain OPVS09
<i>Solanumtuberosum</i>	KJ410668	<i>A. tumefaciens</i> strain KRM13 (KJ124585)	100	<i>A. tumefaciens</i> strain OPVS10
<i>Zea mays</i>	KJ410669	<i>A. tumefaciens</i> strain SWFU-W98 (KF773133)	100	<i>A. tumefaciens</i> strain OPVS11
<i>Saccharumofficinarum</i>	KJ410670	<i>A. tumefaciens</i> strain SWFU-W90 (KF773132)	99	<i>A. tumefaciens</i> strain OPVS12

4.8.2 Effect of KSB on pH dynamics with waste biotite (WB) broth

At 7 DAI, significantly lower pH value was recorded with OPVS-8 followed by OPVS-4. A similar pattern was observed in pH values for OPVS 8 and 6 at 14 and 21 DAI (Fig. 4.7a). Change in pH dynamics also increased with increasing the incubation period of KSB strains. Interaction effects of KSB strains and incubation periods also influenced significantly and lower pH value was recorded at 21 DAI followed by 14 and 7 DAI, respectively (Fig. 4.7). The change in pH dynamics also significantly differed with various KSB strains and production of various organic and inorganic acids.

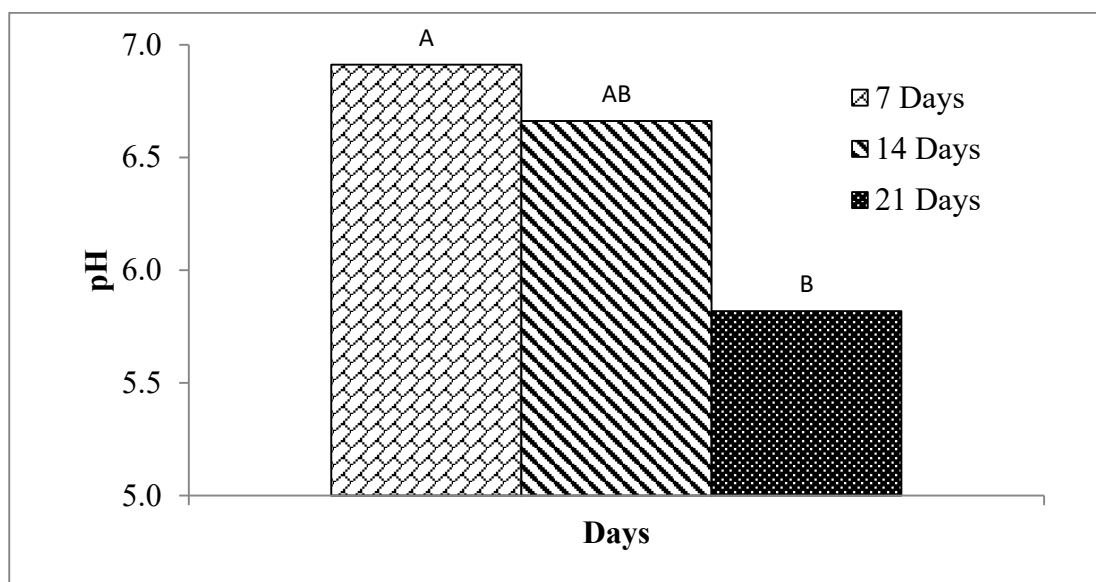


Fig. 4.6a: Interaction effect of KSB on pH with different days of incubation in MAB (Muscovite). Bars followed by a similar letter between treatments within a day of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

Reduction in pH may be due to the production of different kinds of organic and inorganic acids by K-solubilizers. This statement is in accordance with the findings of several workers who have reported that K-solubilizers produce mono-, di- and tri-organic acids, i.e., gluconic, acetic, oxalic, fumaric, tartaric and citric, which resulted in lowering the pH of the spent medium (Girgis *et al.*, 2008; Parmar and Sindhu, 2013). Lowest pH value studies were recorded at 21 DAI in MAB.

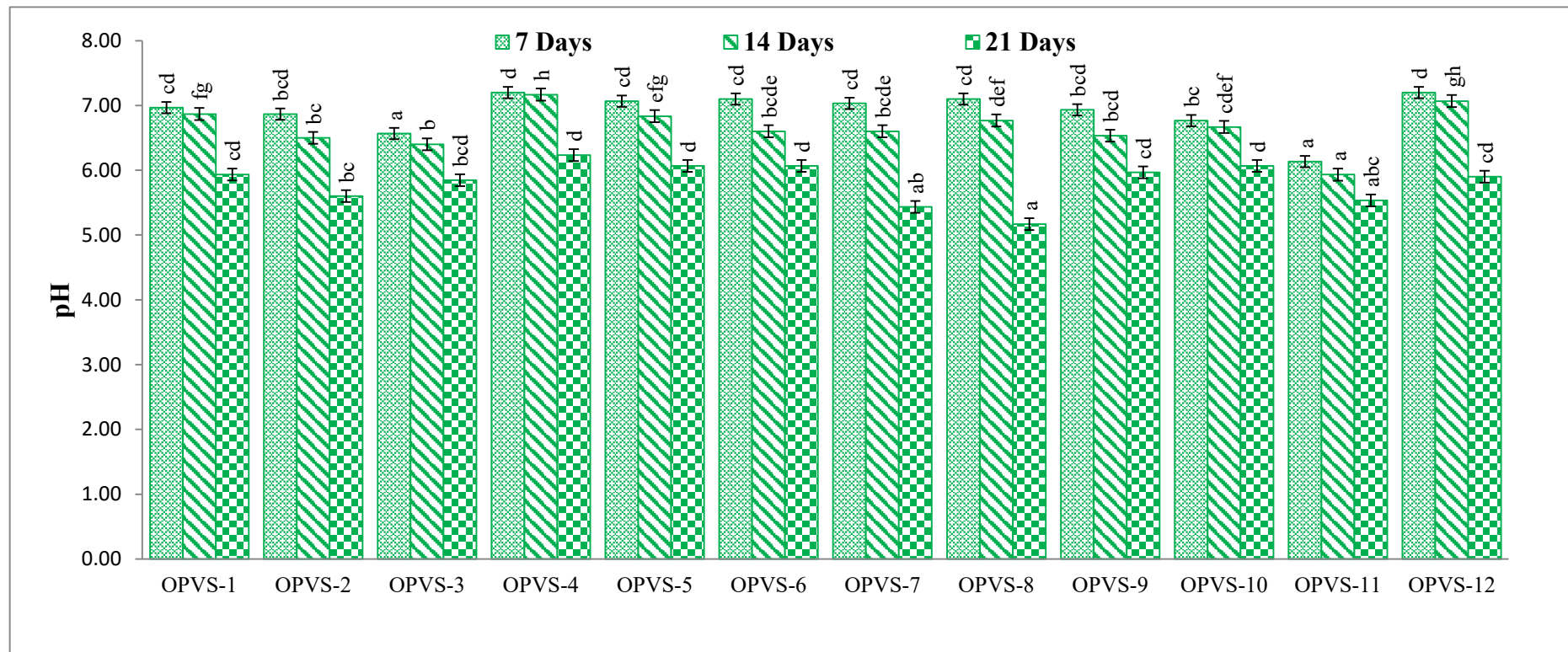


Fig. 4.6b: Effect of KSB on pH of MAB (Muscovite) at different days of inoculation. Each bar represents a mean ($\pm$ SE; $n=3$). Bars followed by a similar letter between treatments within a days of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

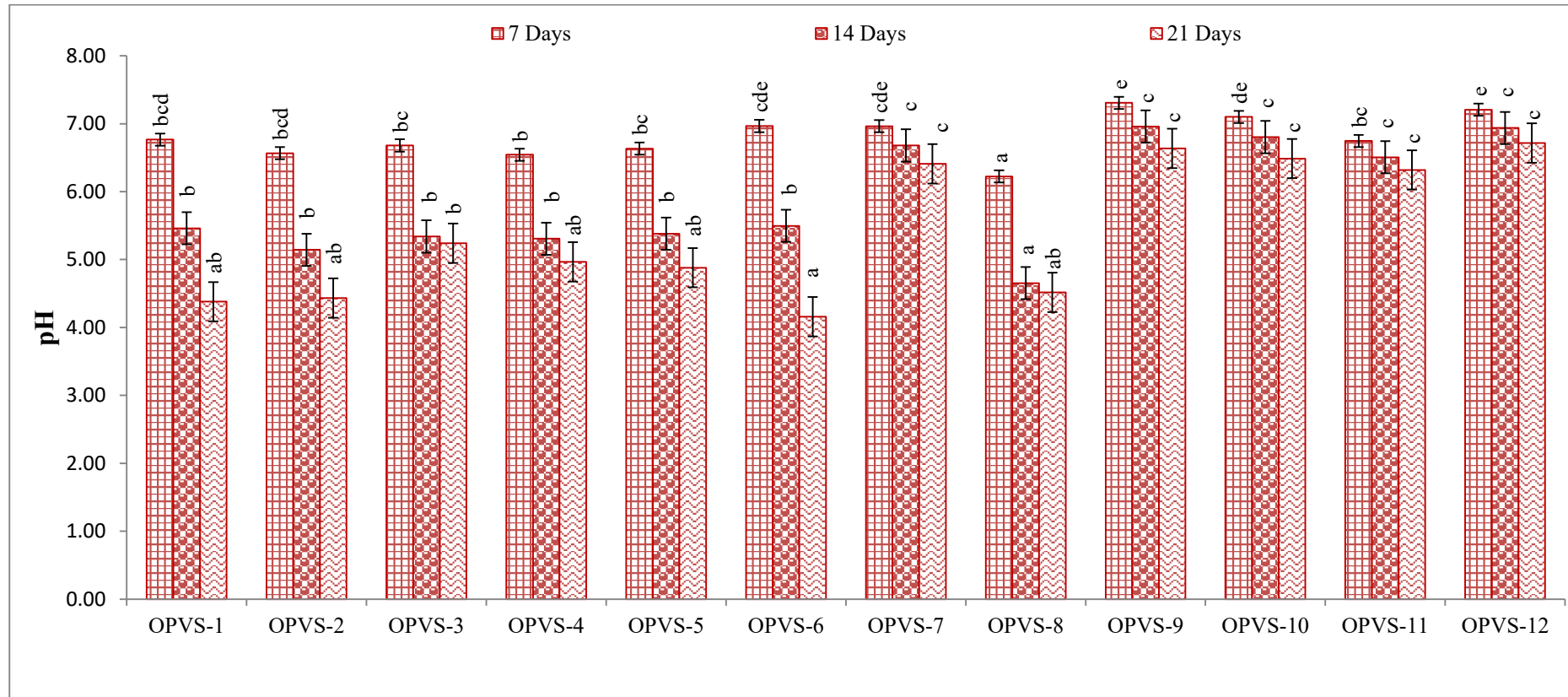


Fig. 4.7a: Effect of KSB on pH of MAB (Biotite) at different days of inoculation Each bar represents a mean ($\pm$ SE; $n=3$). Bars followed by a similar letter between treatments within a days of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.8.3 Effect of KSB on K-solubilization dynamics with WM broth

Very less content of K in uninoculated MAB with waste muscovite was might be due the structural disturbance in waste mica caused by hydrolysis which resulted in the release of K in broth (Zhao *et al.*, 2008; Liu *et al.*, 2012). Organic acids produced by the KSB might have also influenced the frame work destabilizing surface complexes or by complexation metals in solution (Stillings *et al.*, 1996).

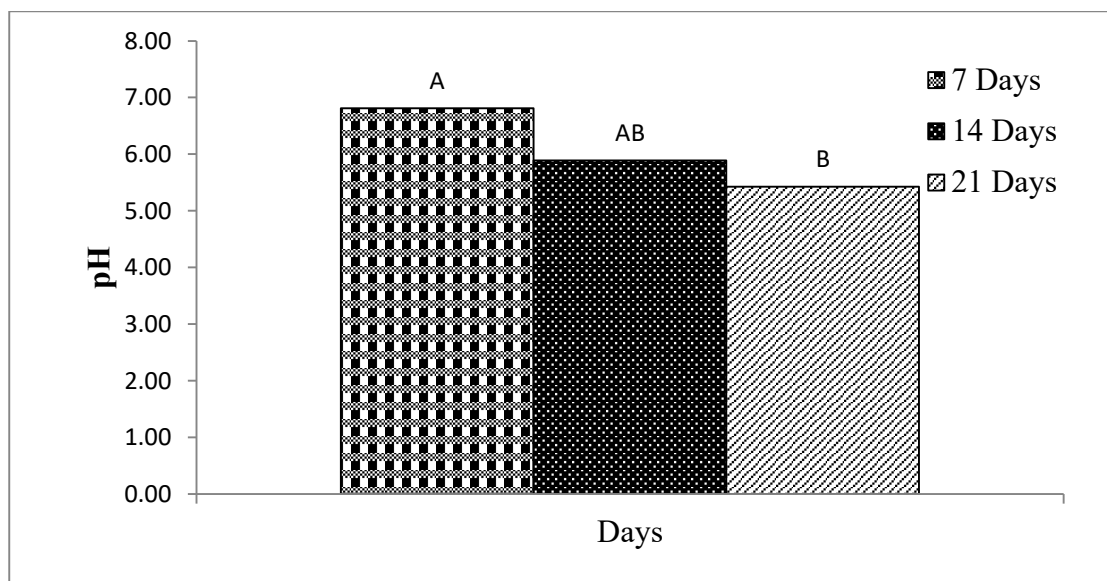


Fig. 4.7b: Interaction effect of KSB on pH with different days of incubation in MAB (Biotite). Bars followed by a similar letter between a day of incubation are not significantly different at $p < 0.05$ level of significance according to DMRT.

The quantity of K solubilization tended to increase with incubation period. Release of K from insoluble waste muscovite was significantly influenced by various KSB strains, incubation periods and their interactions (Fig. 4.8a). All of the KSB strains examined were able to solubilize K but not always to the same extent. In case of waste muscovite, ability of the various KSB strains to solubilize K ranged from 2.86 to 12.86, 6.30 to 11.40 and 9.26 to 16.20 $\mu\text{g/mL}$ at 7, 14 and 21 DAI, respectively (Fig. 4.8b).

Significantly higher K-solubilization was recorded with 21 DAI followed by 14, and 7 DAI. K-solubilization was significantly varied among the various KSB strains with different incubation period, it's depends on the nature of KSB strains like

silicophilic or hydrophilic, when KSB strains have both ability its help to increase the K-solubilizing capacity of KSB strains. Interaction effects of KSB strains with different incubation periods significantly influenced of the solubilization of K significantly higher ($\sim 12 \mu\text{g/mL}$) K-solubilization was recorded with all KSB strains followed by 14 and 7 DAI *in-vitro*.

The K-solubilization potential has been attributed to the KSB strain ability to reduce the pH of the surroundings, either by releasing organic acids or protons (Hariprasad and Niranjana, 2009). Organic acids, such as gluconic acid, oxalic acid, and citric acid, secreted by KSB can directly solubilize mineral phosphate as a result of anion exchange or indirectly chelate both Si and Al ions associated with potassic minerals. This leads to increased K availability, which ultimately increases plant K uptake. It has been reported that bacteria *Bacillus*, *Pseudomonas*, *Rhizobium*, *Burkholderia* and *Pantoea* strains as being efficient potassium solubilizers (Torres *et al.*, 2008; Silini-Cherif *et al.*, 2012; Maurya *et al.*, 2014).

4.8.4 Effect of KSB on K-solubilization dynamics with waste biotite (WB)

Results showed that K-solubilization dynamics of WB significantly influenced by the various KSB strains with different incubation periods. The K-solubilization of different KSB strains with WB minerals ranged from 8.88 to 13.31, 13.25 to 21.60 and 30.34 to 49.73 $\mu\text{g/mL}$ at 7, 14 and 21 DAI, respectively (Fig. 4.9a). Although all KSB strains exhibited significantly more K release as compared to uninoculated broth, and they varied in their capacity to release K from the WB. It is hypothesized that the release of K may be due to the production of different kind of organic acids by the isolates.

The OPVS 1 KSB strain showed significantly higher 49.73 $\mu\text{g/mL}$ K-solubilization capacity, which was significantly superior to compare all other KSB strains. It indicates that KSB strains procured from the same type of soil varied in their capacity of K-solubilization from WB.

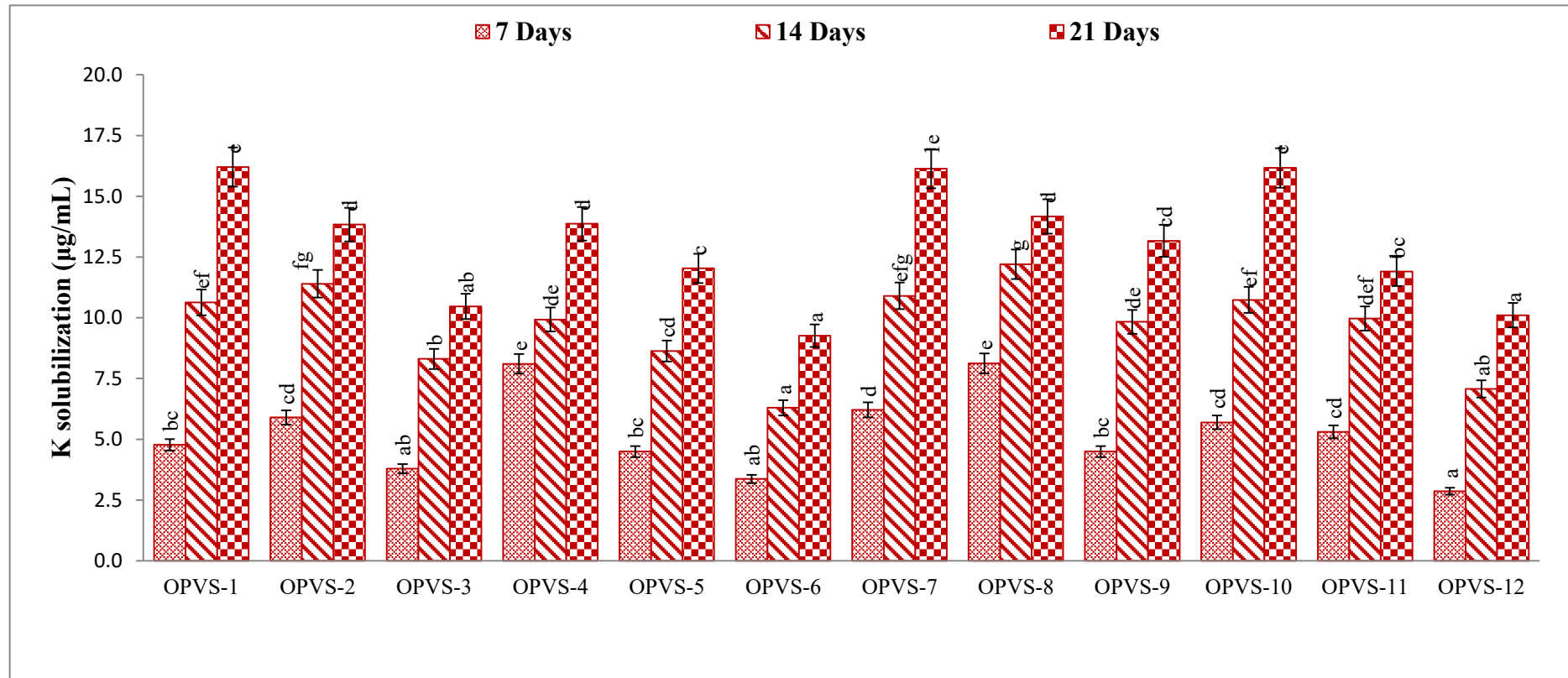


Fig. 4.8a: Potassium solubilization capacity (µg/mL) of KSB in MAB (Muscovite). Each Error bars represent standard errors for three samples from three replications. Bars followed by a similar letter between treatments within a day of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

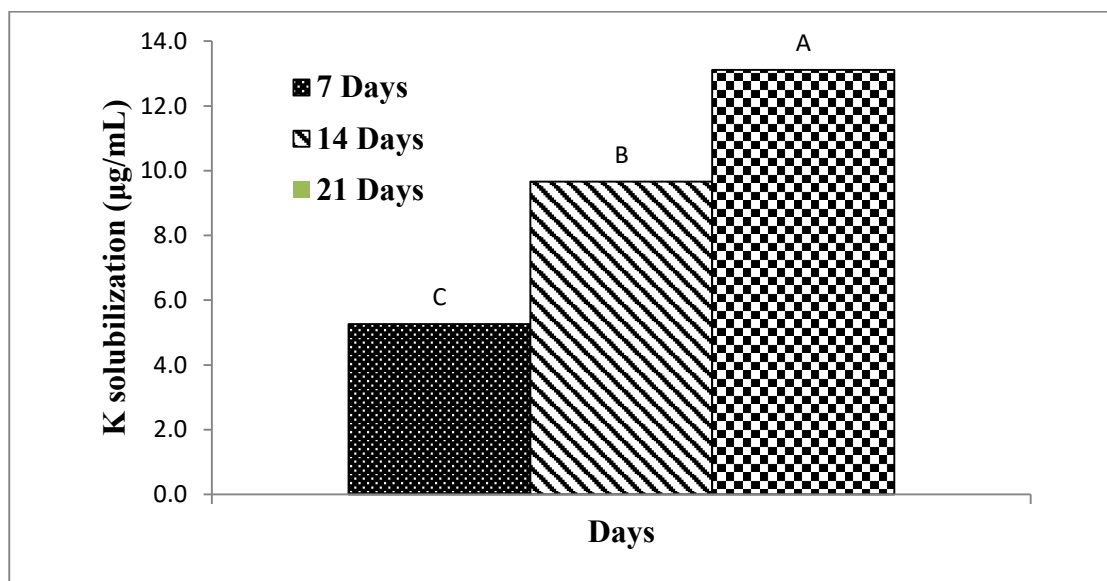


Fig. 4.8b: Interaction effect of KSB on potassium solubilization with different days of incubation in MAB (Muscovite)⁶.

Significantly higher K-solubilization was recorded with 21 DAI followed by 14 and 7 DAI (Fig. 4.9a). Further examination of the solubilization activity of the KSB showed that activity varied and even the same isolate showed different levels of K-solubilization which was consistent with the findings of previous studies who also reported that K-solubilizers produce acids and cause reduction in pH of resulting solution (Caballero-Mellado *et al.*, 2004; Mikhailouskaya and Tcherhysh, 2005). Many workers observed that species of *Bacillus* could solubilize K from K-bearing minerals, which may be attributed to different mechanisms of dissolution of K from these minerals. It was proposed by Vandevivere *et al.* (1994) that *B. mucilaginosus* increases the dissolution rate of silicate and alumino-silicate minerals and releases K^+ and SiO_2 from the crystal lattice primarily due to production of organic acids. It is suggested that the activity of silicate-dissolving bacteria played a crucial role in the release of Si^{+4} , Fe^{+3} and K^+ from feldspar and Fe-oxyhydroxides (Bennett *et al.*, 1998; Styriakova *et al.*, 2004).

⁶bars followed by a similar letter between treatments within a day of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

The association of silicate minerals and bacterial strain can be explained as an outer sphere complex formation as it occurs through electrostatic interactions. The present study indicates that release of K from waste mica (both waste muscovite and biotite) by KSB strains may occur as a result of the action of both EPS and organic acids produced, which can form bi-dentate complexes with metals ions and tend to be more effective in enhancing dissolution than mono-dentate ligands formed by acetate or propionate (Welch and Ullman, 1993).

4.9 Physiological characterization of KSB

4.9.1 Temperature optimization

Results showed that *in-vitro* conditions of the KSB strain cell growth was significantly influenced by different interval of temperature. It was evident that cell growth, increased up to 30°C then after it decreased (Table 4.9). The significantly higher KSB cell growth was recorded with OPVS-9, OPVS-5, 6, 11, OPVS-3, OPVS-3 and OPVS-10 with 20, 25, 30, 35, 40 and 45°C, respectively. Maximum significantly KSB cell growth was recorded with 30°C in all KSB strains. Among them, OPVS-3 had a significantly higher cell growth compared to rest of the KSB strains.

At temperature 20 and 25°C OPVS-9 and OPVS-6 respectively gave higher growth (Table 4.9). After 30°C cell growth decreased order up to 45°C (Fig. 4.10). The highest KSB cell growth was recorded at 30°C which was ~ 66, 26, 22, 49 and 68% with 20, 25, 35, 40 and 45°C, respectively. Temperature variation ($Temp_{max}$) that for KSB growth, from the quadratic response equation, it was estimated that the rate of temperature variation ($Temp_{max}$) that could provide maximum cell growth of KSB. The KSB cell growth ($Temp_{opt}$) is the level of KSB cell growth, where the last increment of temperature just pays for itself, i.e. an increase in KSB cell growth at this point is just sufficient to recover the KSB cell growth and the corresponding variation in temperature is known as the optimum variation rate of temperature ($Temp_{opt}$).

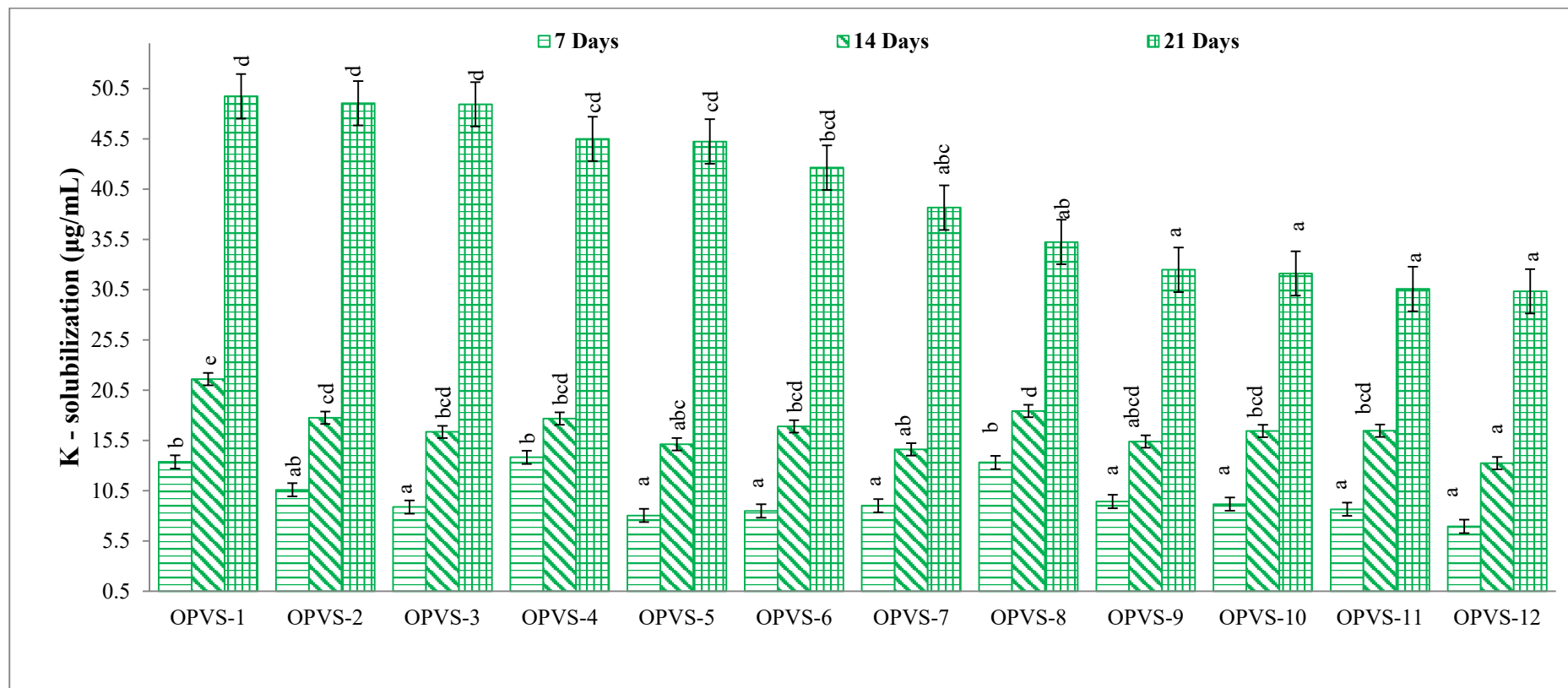


Fig. 4.9a: Potassium solubilization activity (µg/mL) of KSBin MAB (Biotite), Each Error bars represents standard errors for three samples from three replications. Bars followed by a similar letter between treatments within a day of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

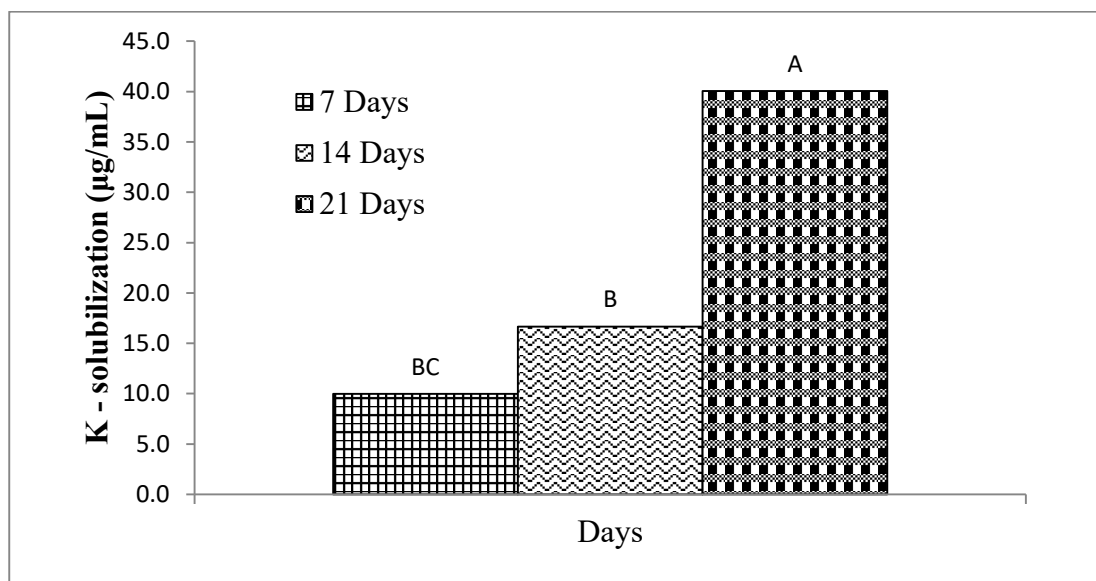


Fig. 4.9b: Interaction effect of KSB on potassium solubilization with different days of incubation in MAB (Biotite), bars followed by a similar letter between treatments within a days of incubation are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

The optimum temperatures were almost the same as the maximum potential temperature for KSB cell growth (Table 4.9). The regression equation for the quadratic response for KSB cell growth with temperature was $y = -0.002\text{Temp.}^2 + 0.147\text{Temp.} - 1.780$, $R^2 = 0.881$ (Fig. 4.10). In addition, PGPR strains are also helpful to alleviate the negative influence of temperature stress (Grichko and Glick, 2001; Babalola *et al.*, 2007; Babalola, 2010).

This indicates that the growth of microorganisms is higher during 21 DAI at 28°C. The bacterial culture showed a variable degree of metabolic effectiveness under different temperatures and incubation periods leading to different degree of utilization of total sugar content in the medium. The lowest sugar content found at 28°C after 21 DAI also reflects the highest metabolic activity of the bacterial culture under this condition. It was found that the metabolic activity of silicate-dissolving bacteria played an important role in the release of Si, Fe and K from silicate minerals by the production of different low molecular organic acids (Styriakova *et al.*, 2004; Biswas and Basak, 2014).

Table 4.9: Effect of temperature intervals on KSB cell growth *in-vitro*

Isolates	20°C	25°C	30°C	35°C	40°C	45°C
OPVS-1	0.237 ± 0.01 ^{abc}	0.445 ± 0.02 ^{cd}	0.652 ± 0.00 ^{bcd}	0.482 ± 0.05 ^d	0.355 ± 0.01 ^d	0.106 ± 0.01 ^f
OPVS-2	0.237 ± 0.02 ^{abc}	0.537 ± 0.04 ^{ab}	0.732 ± 0.03 ^b	0.621 ± 0.02 ^b	0.381 ± 0.03 ^{ab}	0.301 ± 0.01 ^b
OPVS-3	0.203 ± 0.01 ^{cd}	0.447 ± 0.05 ^{cd}	0.854 ± 0.11 ^a	0.701 ± 0.01 ^a	0.402 ± 0.12 ^a	0.209 ± 0.00 ^d
OPVS-4	0.203 ± 0.02 ^{cd}	0.523 ± 0.03 ^{ab}	0.678 ± 0.06 ^{bc}	0.540 ± 0.01 ^c	0.262 ± 0.00 ^e	0.215 ± 0.01 ^d
OPVS-5	0.207 ± 0.02 ^{cd}	0.562 ± 0.04 ^a	0.742 ± 0.04 ^b	0.465 ± 0.02 ^d	0.233 ± 0.02 ^e	0.074 ± 0.01 ^h
OPVS-6	0.246 ± 0.02 ^{ab}	0.575 ± 0.05 ^a	0.687 ± 0.05 ^{bc}	0.471 ± 0.01 ^d	0.298 ± 0.00 ^e	0.088 ± 0.00 ^g
OPVS-7	0.213 ± 0.03 ^{bcd}	0.403 ± 0.04 ^d	0.528 ± 0.04 ^{ef}	0.474 ± 0.02 ^d	0.378 ± 0.09 ^c	0.178 ± 0.01 ^e
OPVS-8	0.193 ± 0.03 ^d	0.338 ± 0.04 ^e	0.519 ± 0.05 ^{ef}	0.460 ± 0.04 ^d	0.315 ± 0.07 ^e	0.184 ± 0.02 ^e
OPVS-9	0.265 ± 0.01 ^a	0.302 ± 0.04 ^e	0.521 ± 0.06 ^{ef}	0.457 ± 0.04 ^d	0.258 ± 0.23 ^e	0.186 ± 0.00 ^e
OPVS-10	0.117 ± 0.02 ^e	0.477 ± 0.03 ^{bc}	0.581 ± 0.01 ^{def}	0.459 ± 0.03 ^d	0.395 ± 0.08 ^a	0.392 ± 0.01 ^a
OPVS-11	0.190 ± 0.02 ^d	0.560 ± 0.02 ^a	0.612 ± 0.04 ^{cde}	0.458 ± 0.04 ^d	0.370 ± 0.14 ^c	0.272 ± 0.00 ^c
OPVS-12	0.220 ± 0.02 ^{bcd}	0.422 ± 0.04 ^{cd}	0.510 ± 0.05 ^f	0.365 ± 0.03 ^e	0.259 ± 0.04 ^e	0.219 ± 0.01 ^d

Data (mean ± SE; n=3) followed by similar letter within a column for a particular temperature interval are not significant different at $p < 0.05$ level of significance according to DMRT.

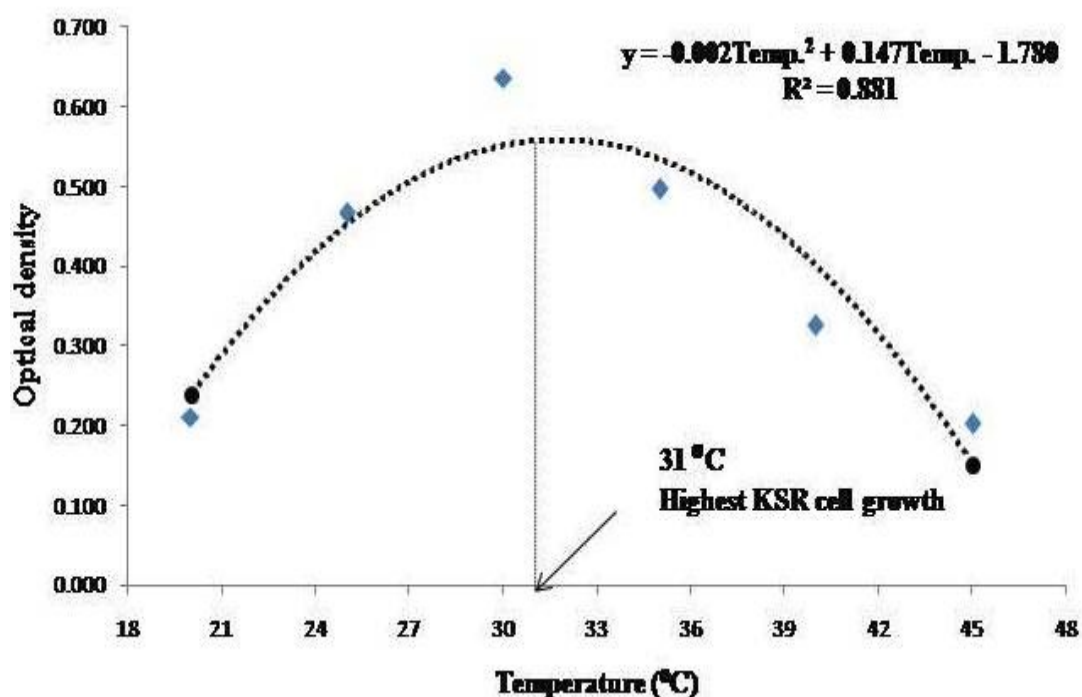


Fig. 4.10. Cell growth of KSB at different temperature *in-vitro* condition

4.9.2 pH optimization

Results showed that variation in pH interval like 6.4, 7.0, 7.6, 8.2, 8.8 and 9.4 significantly influenced the KSB cell growth (Table 4.11). At pH 6.7 significantly higher KSB cell growth was recorded with OPVS-7 and 1 which were showed their significant superiority over rest of the KSB strains.

At pH 7.0 KSB strain OPVS-9 gave significantly higher (4.22 OD) compared to rest of the KSB strain, at pH 7.6 higher value of cell growth was recorded with OPVS-9, at 8.2 pH significantly higher cell growth was recorded with OPVS-5, at pH 8.8 significantly higher cell growth was reported with OPVS-8 and 9 value. However, in case of pH 9.4 significantly higher cell growth was reported with OPVS-8 and 9 (Table 4.11).

KSB cell growth showed significant response to increase or decreased in pH unit under *in-vitro* condition. The highest KSB cell growth was recorded with the 7.6 pH which was ~ 49, 25, 15, 33 and 42% with 6.4, 7.0, 7.6, 8.2, 8.8 and 9.4 pH value,

respectively. The pH variation (pH_{max}) that for KSB growth, from the quadratic response equation, it was estimated that the rate of pH variation (pH_{max}) that could provide maximum cell growth of KSB. The KSB cell growth (pH_{opt}) was the level of KSB cell growth, where the last increment of pH unit just pays for itself, i.e. an increase in KSB cell growth at this point is just sufficient to recover the KSB cell growth and the corresponding variation in pH value is known as the optimum variation rate of pH (pH_{opt}). The optimum temperatures were almost the same as the maximum potential pH unit of the broth culture for KSB cell growth. The regression equation for the quadratic response was KSB cell growth with pH was $y = -0.078\text{pH}^2 + 1.241\text{pH} - 4.485$, $R^2 = 0.838$. The KSB cell growth increased by more than threefold with pH 7.92 treated broth over the rest of the pH of the MAB (Fig. 4.11).

The KSB cell growth was much lower with pH 9.4 with all KMR strains. Although the highest bacterial cell growth was obtained at 7.6 pH adjusted broth (Vahedi, 2013; Nadeem *et al.*, 2014). These results indicated that OPVS-7 is an acid- and OPVS-9 efficient alkali-tolerant bacterium which can be applied to the acidic and alkaline soil, respectively.

4.9.3 Salt optimization

In case of KSB test against different concentration of NaCl salt was added in MAB, it was found that OPVS-8 had significantly higher cell growth in comparison to all other KSB strains at all NaCl concentrations except 10% NaCl (Table 4.11). Significantly higher KSB cell growth was noticed with 1% NaCl concentration followed by 2, 4, 6 and 8% NaCl and significantly lowest KSB cell growth at 10% NaCl concentration.

Table 4.10: Effect of different pH on cell growth of KSB *in-vitro* conditions

Isolates	6.4	7.0	7.6	8.2	8.8	9.4
OPVS-1	0.318 ± 0.02 ^a	0.395 ± 0.02 ^{abc}	0.506 ± 0.01 ^a	0.465 ± 0.02 ^{ab}	0.377 ± 0.02 ^a	0.254 ± 0.01 ^{def}
OPVS-2	0.326 ± 0.03 ^a	0.395 ± 0.03 ^{abc}	0.472 ± 0.02 ^{ab}	0.330 ± 0.02 ^f	0.285 ± 0.02 ^{cd}	0.222 ± 0.01 ^g
OPVS-3	0.229 ± 0.02 ^{cd}	0.338 ± 0.01 ^{cd}	0.430 ± 0.02 ^b	0.384 ± 0.01 ^{de}	0.323 ± 0.01 ^b	0.245 ± 0.01 ^{efg}
OPVS-4	0.192 ± 0.02 ^{de}	0.338 ± 0.03 ^{cd}	0.438 ± 0.39 ^b	0.355 ± 0.01 ^{ef}	0.256 ± 0.01 ^{de}	0.216 ± 0.00 ^g
OPVS-5	0.171 ± 0.02 ^e	0.345 ± 0.03 ^{cd}	0.533 ± 0.04 ^a	0.487 ± 0.01 ^a	0.262 ± 0.02 ^{de}	0.230 ± 0.04 ^{fg}
OPVS-6	0.270 ± 0.02 ^b	0.410 ± 0.03 ^{ab}	0.530 ± 0.01 ^a	0.445 ± 0.01 ^{bc}	0.312 ± 0.02 ^{bc}	0.265 ± 0.02 ^{cde}
OPVS-7	0.318 ± 0.01 ^a	0.355 ± 0.05 ^{bcd}	0.441 ± 0.06 ^b	0.395 ± 0.04 ^d	0.247 ± 0.02 ^e	0.180 ± 0.02 ^h
OPVS-8	0.239 ± 0.02 ^{bc}	0.322 ± 0.04 ^d	0.461 ± 0.01 ^b	0.444 ± 0.01 ^{bc}	0.402 ± 0.01 ^a	0.395 ± 0.02 ^a
OPVS-9	0.181 ± 0.03 ^e	0.442 ± 0.02 ^a	0.528 ± 0.02 ^a	0.413 ± 0.02 ^{cd}	0.385 ± 0.00 ^a	0.376 ± 0.01 ^a
OPVS-10	0.130 ± 0.02 ^f	0.195 ± 0.03 ^e	0.411 ± 0.01 ^b	0.341 ± 0.01 ^f	0.301 ± 0.04 ^{bc}	0.293 ± 0.01 ^{bc}
OPVS-11	0.237 ± 0.02 ^{bc}	0.317 ± 0.03 ^d	0.422 ± 0.03 ^b	0.352 ± 0.03 ^{ef}	0.329 ± 0.02 ^b	0.314 ± 0.01 ^b
OPVS-12	0.208 ± 0.02 ^{cde}	0.367 ± 0.03 ^{bcd}	0.434 ± 0.10 ^b	0.352 ± 0.01 ^{ef}	0.311 ± 0.01 ^{bc}	0.283 ± 0.02 ^{bcd}

Data (mean ± SE; n=3) followed by similar letter within a column for a particular pH interval are not significantly different at p < 0.05 level of significance according to DMRT (for KSB strain detail see the methods and material section)

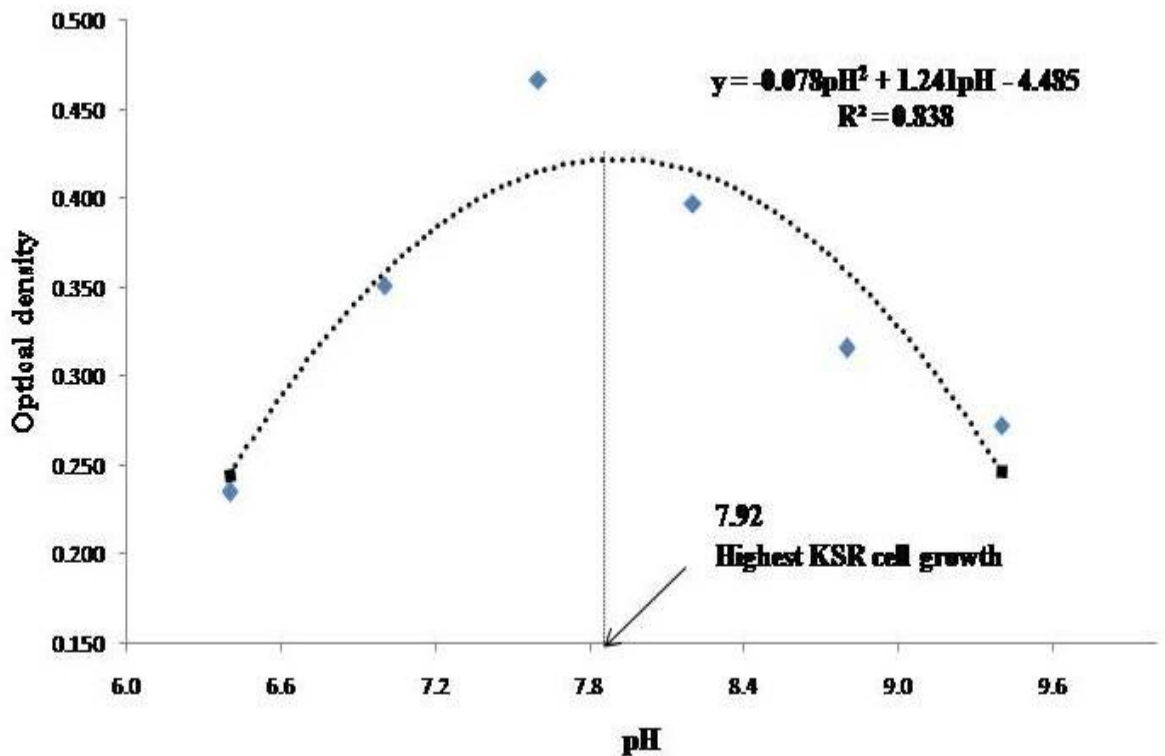


Fig. 4.11: Response of pH levels to KSB cell growth *in-vitro* condition

All KSB strains decreased cell growth with increasing salt concentration the regression equation for the quadratic response was KSB cell growth $y = 0.000NaCl^2 - 0.037NaCl + 0.571$, $R^2 = 0.972$ (Fig. 4.12). KSB growth showed significant response to increase concentration of NaCl under *in-vitro* condition. The highest KSB cell growth was recorded with the 1% NaCl, which was ~ 14, 25, 30, 40 and 52% with 2, 4, 6, 8 and 10% NaCl, respectively.

NaCl concentration ($NaCl_{max}$) that for KSB growth, from the quadratic response equation, it was estimated that the rate of NaCl concentration ($NaCl_{max}$) that could provide maximum cell growth of KSB (Table 4.11). The KSB cell growth ($NaCl_{opt}$) is the level of KSB cell growth, where the last increment of NaCl concentration just pays for itself, i.e. an increase in KSB cell growth at this point is just sufficient to recover the KSB cell growth and the corresponding variation in NaCl concentration is known as the optimum variation rate of NaCl ($NaCl_{opt}$). The optimum NaCl concentrations was almost same as the maximum potential NaCl concentration for KSB cell growth (Table 4.12).

Table 4.11: Effect of salt concentration (NaCl) on potassium solubilizing bacterial (KSB) cell growth *in-vitro* conditions

Isolates	1 % NaCl	2 % NaCl	4 % NaCl	6 % NaCl	8 % NaCl	10 % NaCl
OPVS-1	0.387 ± 0.06 ^h	0.321 ± 0.04 ^d	0.221 ± 0.01 ^f	0.213 ± 0.02 ^g	0.179 ± 0.07 ^e	0.143 ± 0.04 ^e
OPVS-2	0.551 ± 0.04 ^{ef}	0.468 ± 0.03 ^c	0.310 ± 0.08 ^e	0.254 ± 0.05 ^g	0.222 ± 0.03 ^{de}	0.191 ± 0.01 ^{cd}
OPVS-3	0.412 ± 0.03 ^h	0.348 ± 0.02 ^d	0.334 ± 0.01 ^{de}	0.336 ± 0.02 ^{ef}	0.282 ± 0.05 ^{cd}	0.164 ± 0.02 ^{de}
OPVS-4	0.622 ± 0.04 ^{cd}	0.498 ± 0.02 ^{bc}	0.457 ± 0.01 ^{bc}	0.451 ± 0.03 ^{bc}	0.417 ± 0.03 ^{ab}	0.358 ± 0.01 ^a
OPVS-5	0.469 ± 0.03 ^g	0.405 ± 0.02 ^{cd}	0.387 ± 0.01 ^d	0.381 ± 0.02 ^{de}	0.381 ± 0.01 ^{ab}	0.365 ± 0.02 ^a
OPVS-6	0.503 ± 0.01 ^{fg}	0.469 ± 0.07 ^c	0.369 ± 0.03 ^{de}	0.316 ± 0.05 ^f	0.284 ± 0.03 ^{cd}	0.226 ± 0.03 ^c
OPVS-7	0.510 ± 0.01 ^{fg}	0.473 ± 0.06 ^c	0.445 ± 0.01 ^c	0.438 ± 0.03 ^{bcd}	0.373 ± 0.07 ^{ab}	0.221 ± 0.01 ^c
OPVS-8	0.729 ± 0.04 ^a	0.665 ± 0.03 ^a	0.580 ± 0.01 ^a	0.559 ± 0.03 ^a	0.414 ± 0.01 ^{ab}	0.213 ± 0.01 ^c
OPVS-9	0.661 ± 0.01 ^{bc}	0.486 ± 0.13 ^{bc}	0.509 ± 0.02 ^b	0.495 ± 0.03 ^b	0.348 ± 0.00 ^{bc}	0.334 ± 0.01 ^a
OPVS-10	0.700 ± 0.05 ^{ab}	0.584 ± 0.07 ^{ab}	0.503 ± 0.01 ^{bc}	0.494 ± 0.02 ^{bc}	0.428 ± 0.02 ^{ab}	0.349 ± 0.04 ^a
OPVS-11	0.534 ± 0.01 ^{ef}	0.497 ± 0.08 ^{bc}	0.467 ± 0.07 ^{bc}	0.411 ± 0.05 ^d	0.380 ± 0.01 ^{ab}	0.345 ± 0.03 ^a
OPVS-12	0.578 ± 0.03 ^{de}	0.460 ± 0.04 ^c	0.343 ± 0.01 ^{de}	0.328 ± 0.02 ^{ef}	0.304 ± 0.06 ^c	0.280 ± 0.01 ^b

Data (mean ± SE; n=3) followed by similar letter within a column for a particular salt concentration are not significant different at p< 0.05 level of significance according to DMRT.

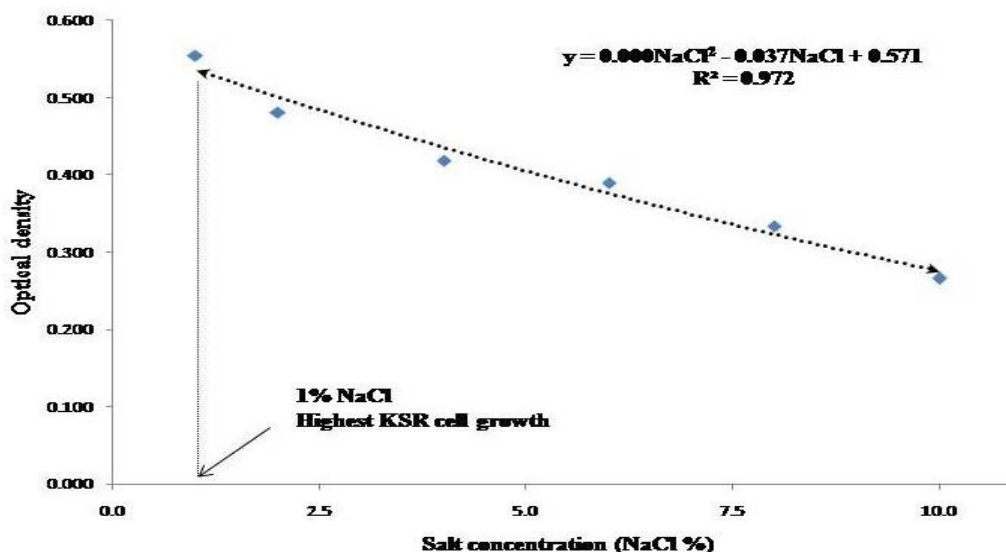


Fig. 4.12: Response of NaCl concentrations to KSB cell growth *in-vitro* condition

Son *et al.* (2006) worked on the bacteria which is able to solubilize insoluble minerals under high saline environment. These strains demonstrated high level WM and WB mineralization under stress conditions, temperature (20 - 45°C), pH (6.4 – 9.4) and NaCl (1 - 10%). KSB strain showed changes in growth pattern under high salt and temperature conditions as compared to control (Fig. 4.12). However, when relieved from the stress conditions it regained control conditions (Kulkarni and Nautiyal, 1999).

4.10 Multivariate analysis

4.10.1 Principal component analysis (PCA) for waste muscovite

PCA is a useful statistical technique which had found application in reduction of the original variables in a smaller number of underlying variables (principal component) in order to explain the interrelationships between the different variables and to find the optimum number of extracted principal components. KSB under different stress condition, K-solubilization from waste muscovite, and changes in pH dynamics were depicted in Fig. 4.13a. The PCA comprising two principal components (C1 and C2) accounted for ~ 53 of the variance for KSB with different growth conditions. An angle of 0 or 180° reflects a correlation of 1 or -1, respectively (Kohler and Luniak, 2005).

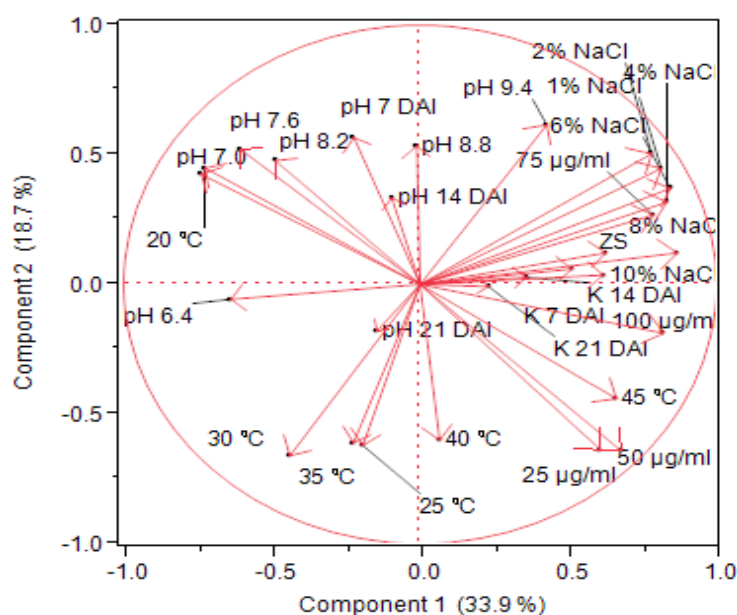


Fig. 4.13a: Loading plot of multivariate factorial comparison of different K-solubilizing activities using PCA with muscovite as a sole source of potassium

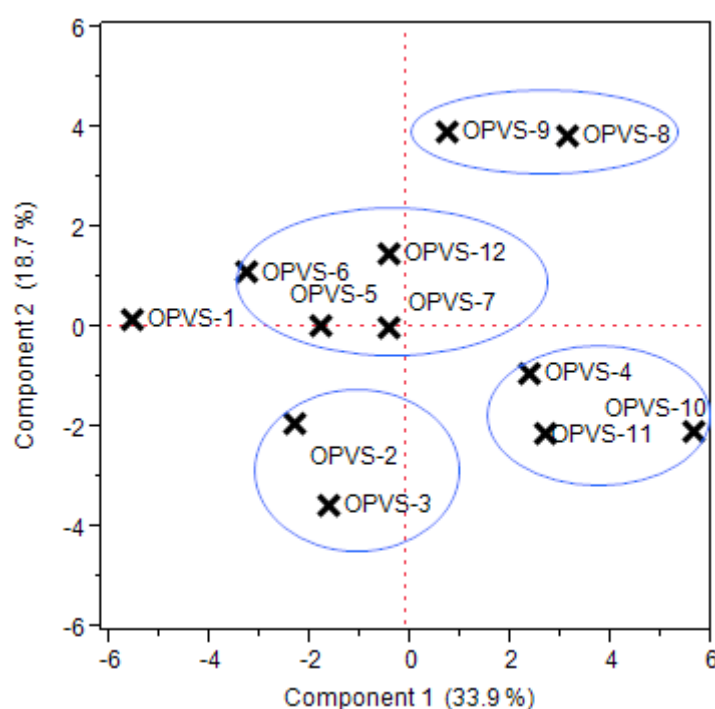


Fig. 4.13b: Grouping of KSB, based on principal component scores with muscovite as a sole source of potassium (see the materials and methods section for strains details).

Superimposition of KSB and K-solubilization from waste muscovite with different stress growth environment showed that OPVS-5, 6, 7 and 12 showed a higher correlation with these parameters (Tables 4.12 and 4.13). The PCA of K-solubilization activities from waste muscovite comprising two principal components (C133.9; C2 18.7% for KSB) accounted for ~ 53 of variance (Fig. 13a). The C1 and C2 had a cluster of KSB (*R. rosettiformans* strain OPVS04, *A. tumefaciens* strain OPVS10 and *A. tumefaciens* strain OPVS11) with large positive loading for the first component. K-solubilization activities from waste muscovite and the pH had strongly negative loading for first and second component for KSB.

A position of 29 parameters in relation to their influence by K-solubilization activities from waste muscovite accounted from ~ 53% variants for KSB, the interpretation of the PCA results can be explained by the positioning of the KSB strain group and group of K-solubilization activities from waste muscovite by superimposition of respective PCA plots for respective KSB strains. As a result of cluster analysis, three clusters were obtained.

Table 4.12: Composition of clusters of 11 KSB activities with muscovite based on value of principal component analysis (PCA)

Cluster no.	No. of KSB	Name of KSB species
I	3	<i>R. rosettiformans</i> strain OPVS04
		<i>A. tumefaciens</i> strain OPVS10
		<i>A. tumefaciens</i> strain OPVS11
II	2	<i>F. anhuiense</i> strain OPVS08
		<i>A. tumefaciens</i> strain OPVS09
III	2	<i>A. tumefaciens</i> strain OPVS02
		<i>R. pusense</i> strain OPVS03
		<i>F. anhuiense</i> strain OPVS05
IV	4	<i>R. pusense</i> strain OPVS06
		<i>A. tumefaciens</i> strain OPVS07
		<i>A. tumefaciens</i> strain OPVS12

The cluster I possessed KSB of *R. rosettiformans* strain OPVS04, *A. tumefaciens* strain OPVS10 and *A. tumefaciens* strain OPVS11 (Tables 4.12 and 4.13), which are best discernible by their highest mean values of positive influencing K-solubilization activities from waste muscovite and lowest mean values for negative

influencing K-solubilization activities from waste muscovite. The next best cluster was II, which retained *F. anhuiense* strain OPVS08 and *A. tumefaciens* strain OPVS09, followed by cluster III with *A. tumefaciens* strain OPVS02 and *R. pusense* strain OPVS03 and cluster IV which retained *F. anhuiense* strain OPVS05, *R. pusense* strain OPVS06, *A. tumefaciens* strain OPVS07 and *A. tumefaciens* strain OPVS12 (Tables 4.12 and 4.13).

4.10.2 Principal component analysis (PCA) for waste biotite

PCA is a useful statistical technique which had found application in reduction of the original variables in a smaller number of underlying variables (principal component) in order to reveal the interrelationships between the different variables and to find the optimum number of extracted principal components. KSB under different stress condition, K-solubilization from waste biotite, and changes in pH dynamics were depicted in Fig. 4.14a. The PCA comprising two principal components (C1 and C2) accounted for ~ 55% of the variance for KSB with different growth conditions. An angle of 0 or 180° reflects a correlation of 1 or -1, respectively (Kohler and Luniak, 2005). Superimposition of KSB and K-solubilization from waste biotite with different stress growth environment showed that OPVS-5, 6 and 7 showed a higher correlation with these parameters (Tables 4.14 and 4.15).

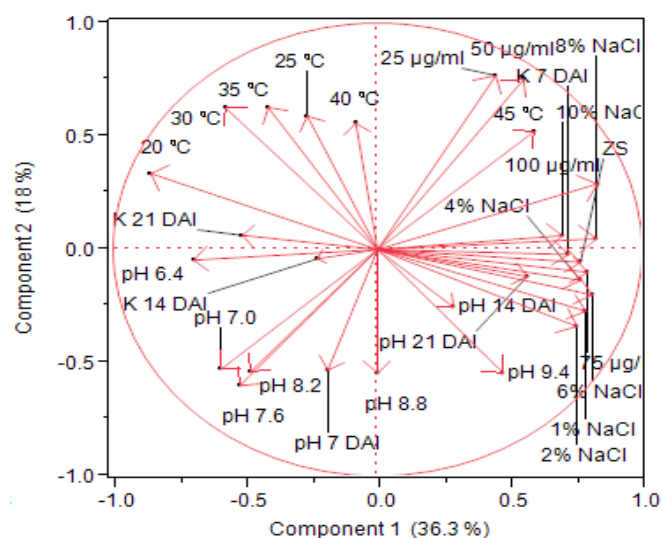


Fig. 4.14a: Loading plot of multivariate factorial comparison of different K-solubilizing activities using PCA with biotite as a sole source of potassium.

Table 4.13: Mean values of potassium solubilizing activities with muscovite of the culture broth of four clusters of KSB

Potassium solubilizing activities	Clusters			
	I	II	III	IV
Zone of solubilization (cm)	2.22	2.18	1.68	2.14
IAA production with 25 µg/mL tryptophan	8.12	6.81	6.61	5.54
IAA production with 50 µg/ mL tryptophan	9.47	8.53	7.95	7.35
IAA production with 75 µg/ mL tryptophan	13.25	12.92	11.25	11.84
IAA production with 100 µg/ mL tryptophan	19.89	19.65	16.70	15.42
Modified Aleksandrov broth (MAB) pH 7 DAI	6.70	6.85	6.72	7.10
MAB pH 14 DAI	6.59	6.60	6.45	6.78
MAB pH 21 DAI	5.94	6.02	5.72	5.87
K solubilization 7 DAI (µg/ mL)	6.37	5.10	4.85	4.23
Ksolubilization 14 DAI (µg/ mL)	10.21	10.28	9.85	8.23
Ksolubilization 21 DAI (µg/ mL)	13.98	14.67	12.15	11.89
20°C	0.17	0.19	0.22	0.22
25°C	0.52	0.39	0.49	0.49
30°C	0.62	0.55	0.79	0.62
35°C	0.49	0.46	0.66	0.44
40°C	0.34	0.33	0.39	0.29
45°C	0.29	0.29	0.26	0.14
pH 6.4	0.19	0.16	0.28	0.24
pH 7.0	0.28	0.32	0.37	0.37
pH 7.6	0.42	0.47	0.45	0.48
pH 8.2	0.35	0.38	0.36	0.42
pH 8.8	0.30	0.34	0.30	0.28
pH 9.4	0.27	0.33	0.23	0.24
1% NaCl	0.62	0.68	0.48	0.52
2% NaCl	0.53	0.58	0.41	0.45
4% NaCl	0.48	0.51	0.37	0.39
6% NaCl	0.45	0.49	0.29	0.37
8% NaCl	0.41	0.39	0.25	0.34
10% NaCl	0.35	0.34	0.18	0.27

The PCA of K-solubilization activities from waste biotite comprising two principal components (C1 36.3; C2 18% for KSB) accounted for ~ 55% of variance (Fig. 4.14a). The C1 and C2 had a cluster of KSB (*A. tumefaciens* strain OPVS02 and *R. pusense* strain OPVS03) with large positive loading for the first component. K-solubilization activities from waste biotite and the pH had strongly negative loading for first and second component for KSB. A position of 29 parameters in relation to their influence by K-solubilization activities from waste biotite accounted from ~ 55% variants for KSB, the interpretation of the PCA results can be explained by the positioning of the KSB strain group and group of K-solubilization activities from waste biotite by superimposition of respective PCA plots for respective KSB strains.

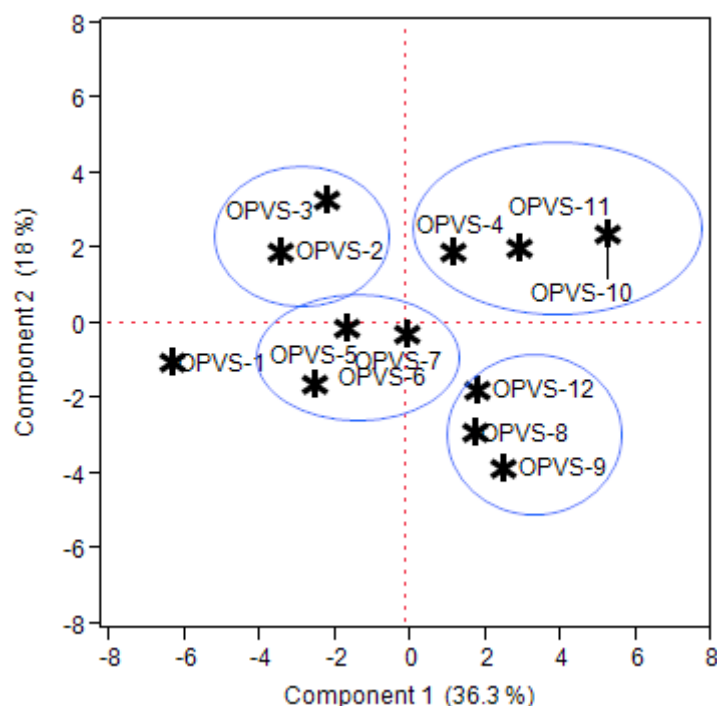


Fig. 4.14b: Grouping of KSB based on principal component scores with biotite as a sole source of potassium (see the materials and methods section for strains details).

As a result of cluster analysis, three clusters were obtained. The cluster I possessed KSB of *A. tumefaciens* strain OPVS02 and *R. pusense* strain OPVS03, which are best discernible by their highest mean values of positive influencing K-solubilization activities from waste biotite and lowest mean values for negative influencing K-solubilization activities from waste biotite. The next best cluster was II, which retained *R. rosettiformans* strain OPVS04, *A. tumefaciens* strain OPVS10, and

A. tumefaciens strain OPVS11, followed by cluster III with *F. anhuiense* strain OPVS05, *R. pusense* strain OPVS06 and *A. tumefaciens* strain OPVS07 and cluster IV which retained *F. anhuiense* strain OPVS08, *A. tumefaciens* strain OPVS09 and *A. tumefaciens* strain OPVS12 (Tables 4.14 and 4.15).

Table 4.14: Composition of clusters of 11 KSB activities with biotite based on value of principal component analysis (PCA)

Cluster no.	No. of KSB	Name of KSB species
I	2	<i>A. tumefaciens</i> strain OPVS02 <i>R. pusense</i> strain OPVS03
II	3	<i>R. rosettiformans</i> strain OPVS04 <i>A. tumefaciens</i> strain OPVS10 <i>A. tumefaciens</i> strain OPVS11
III	3	<i>F. anhuiense</i> strain OPVS05 <i>R. pusense</i> strain OPVS06 <i>A. tumefaciens</i> strain OPVS07
IV	3	<i>F. anhuiense</i> strain OPVS08 <i>A. tumefaciens</i> strain OPVS09 <i>A. tumefaciens</i> strain OPVS12

4.11 Effect of KSB strains on seed germination

4.11.1 Seed germination

Seed inoculation with KSB strains significantly enhanced seed germination, (Fig. 4.16). However, the rate of enhancement varied with the potassium solubilizing bacterial (KSB) strains. The results of germination of seed significantly influenced by the different KSB strains *in-vitro* condition (Table 4.17). In case of the seed germination, shoot and root length of maize seedlings varied significantly with various strains of KSB.

The seed germination was significantly influenced by the different KSB strains. Results showed that the germination varied from 85.89 to 95.49% with various KSB strains. Significantly higher (95.49%) seed germination was recorded with KSB strains OPVS-3; it was ~ 11% higher as compared to uninoculated pot treatment (85.89%).

Table 4.15: Mean values of potassium solubilizing activities with biotite of the culture broth of four clusters of KSB

Potassium solubilizing activities	Clusters			
	I	II	III	IV
Zone of solubilization (cm)	1.68	2.22	2.13	2.10
IAA production with 25 µg/ mL tryptophan	6.61	8.12	5.64	5.32
IAA production with 50 µg/ mL tryptophan	7.95	9.47	7.47	7.11
IAA production with 75 µg/ mL tryptophan	11.25	13.25	11.33	13.28
IAA production with 100 µg/ mL tryptophan	16.70	19.89	14.53	18.07
Modified Aleksandrov broth (MAB) pH 7 DAI	6.72	6.70	7.07	7.08
MAB pH 14 DAI	6.62	6.80	6.85	6.91
MAB pH 21 DAI	5.24	6.21	5.85	6.18
K solubilization 7 DAI (µg/ mL)	4.84	5.92	5.15	5.96
Ksolubilization 14 DAI (µg/ mL)	9.74	10.55	8.51	9.90
Ksolubilization 21 DAI (µg/ mL)	17.06	16.86	15.55	15.69
20°C	48.99	36.06	42.18	32.68
25°C	0.49	0.52	0.51	0.35
30°C	0.79	0.62	0.65	0.52
35°C	0.66	0.49	0.47	0.43
40°C	0.39	0.34	0.30	0.28
45°C	0.26	0.29	0.11	0.20
pH 6.4	0.28	0.19	0.25	0.21
pH 7.0	0.37	0.28	0.37	0.38
pH 7.6	0.45	0.42	0.50	0.47
pH 8.2	0.36	0.35	0.44	0.40
pH 8.8	0.30	0.30	0.27	0.37
pH 9.4	0.23	0.27	0.23	0.35
1% NaCl	0.48	0.62	0.49	0.66
2% NaCl	0.41	0.53	0.45	0.57
4% NaCl	0.37	0.48	0.40	0.48
6% NaCl	0.29	0.45	0.38	0.46
8% NaCl	0.25	0.41	0.35	0.36
10% NaCl	0.18	0.35	0.27	0.28

The OPVS-3 KSB showed significant superiority over the rest of the KSB stain and control pot, except strain OPVS-6 and OPVS-10 (Fig. 4.17). The seed germination of maize crops was significantly varied among the KSB strains and recorded in the following order OPVS-3, the seed germination found in order of $OPVS-6 \geq OPVS-10, > OPVS-4, > OPVS-2, OPVS-12, OPVS-1, OPVS-9, OPVS-11, OPVS-7, OPVS-8, OPVS-5$ and control pot. KSB inoculation of seed enhances the seed germination by producing plant hormones as secondary metabolites. These hormones improved germination of maize seed inoculated with different KSB isolates (Miransari and Smith, 2014).

4.11.2 Shoot and root length

The KSB strains significantly increased the shoot and root length of maize seedlings *in-vitro* condition as compared to uninoculated (Figs. 4.17 and 4.18). The significantly higher shoot and root length 15.56 and 13.58 cm, respectively were recorded with inoculation with KSB OPVS-10 strain and it was ~ 4 fold higher as compared to uninoculated control pot followed by the OPVS-5 KSB strain (13.99 cm). Promotaion of root length and consequently the shoot length was may be affected to the production of IAA by KSB isolates growth. Further growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Kumar *et al.*, 2015).

Data on shoot length showed that the KSB strain OPVS-11 caused its significantly higher value (12.90 cm) compared to rest the KSB strains. In terms of percentage it was (~ 74%) more than control pot (Fig. 4.17). All twelve KSB strains capable of producing phytohormone such as IAA, siderophore and solubilization of mineral phosphate exerts direct impact on plant growth through modification in root morphology, proliferation and increase the nutrient availability to the host plants. All twelve KSB strains was produced of phytohormone IAA in varied amounts. Improvement in root and shoot length may be due to an increase of the synthesis of the hormone gibberellic acid, which involved in hydrolysis and assimilation of the starch (Gholami *et al.*, 2009; Kumar *et al.*, 2014).

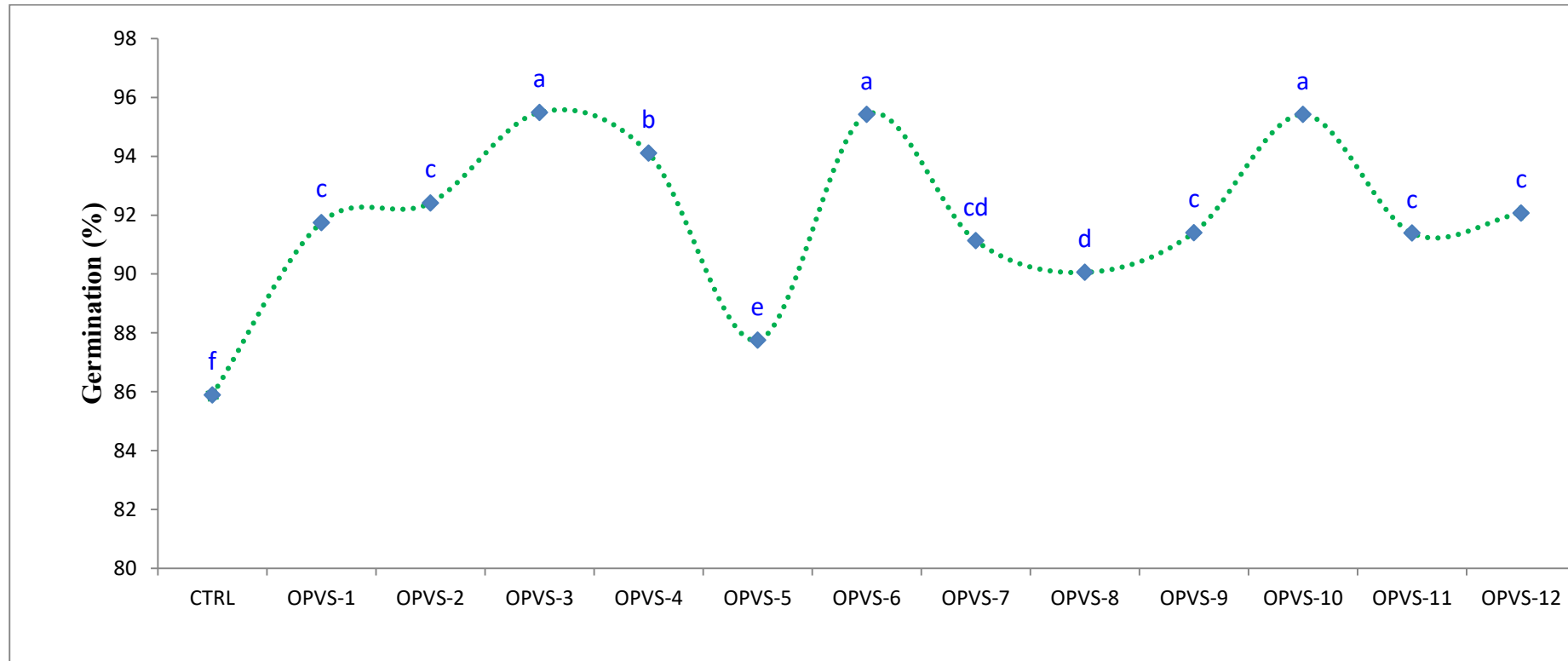


Fig. 4.16: Effect of various KSB strains on seed germination under net house conditions. Data are presented as mean $\pm$ standard error (n = 3), Mean followed by similar letter within a column for a particular parameter are not significantly different ($P < 0.05$) level of significance according to DMRT.

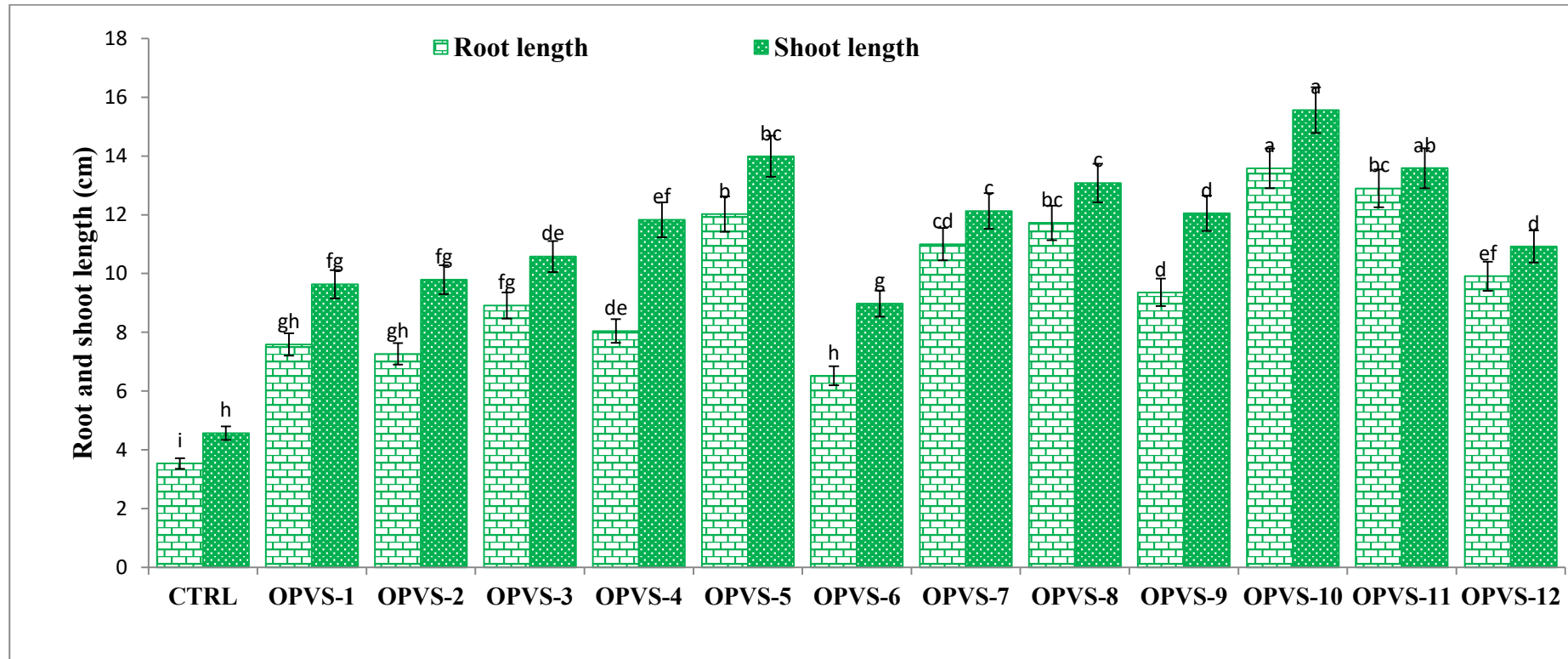


Fig. 4.17: Effect of various KSB strains on shoot and root length under net house conditions. Data are presented as mean $\pm$ standard error ($n = 3$), Mean followed by similar letter within a column for a particular parameter are not significantly different ($P < 0.05$) level of significance according to DMRT.



Fig. 4.18: Effect of different KSB strains on root and shoot length of maize crop after 10 DAS *in-vitro* condition.

4.12 Effect of various KSB strains and levels of K fertilization on growth attributes of maize under net house condition

4.12.1 Plant height

The data pertaining to plant height at 60 days after sowing (DAS) as influenced by different KSB and levels of K fertilization have been presented in Table 4.17. Increase in plant height of maize was observed with various KSB strains and levels of potassium fertilization as compared to control pot. The increase in plant height significantly differed with the potassium solubilizing bacterial (KSB) strains.

Significantly higher plant height (156.01 cm) of maize crop at 60 DAS with the application of 100% RDK was observed with KSB OPVS-10 strain and it was significantly superior to the rest of KSB strains. OPVS-10 KSB strain produced ~ 27% higher plant height than OPVS-1 (122.77 cm) and 26.88% greater than control (122.96 cm). Strain OPVS-11 gave 147.25 cm plant height that was 19.75% higher than control. It was worth to note that with 100% RDK with OPVS-1 have (122.77 cm) of plant height that was lesser than control pot but variation between them was statically insignificant.

With the application of 75% RDK, maximum plant height (145.42 cm) was recorded with OPVS-10 treated pot. In terms of percent, it was 29.53% more than control. All the isolates gave greater height of plant in comparison to uninoculated control with doses of potassium. In terms of plant height KSB strains performed in order of OPVS-10 > OPVS-11 > OPVS-7 > OPVS-6 > OPVS-4 > OPVS-12 > OPVS-5 > OPVS-3 > OPVS-8 > OPVS-2 > OPVS-9 > OPVS-1 and control pots (Table 4.16).

However, in case of 50% RDK in comparison to 75 and 100% RDK relatively lower plant height was observed, but on other hand KSB strain shows higher efficiency in terms of percent increase in plant height than control pot. The highest plant height (137.41 cm) was observed with KSB OPVS-10 strain that was 32.35% higher over the control pot (103.82 cm). OPVS-10 strain was significantly superior to rest of the KSB strains. Strain OPVS-6 and OPVS-11 caused 132.94 and 132.72 cm

of plant height of maize crop. All the isolates significantly more height of maize crop when compared with control.

Table 4.16: Effect of different KSB strains and levels of potassium on plant height (cm) at 60 DAS of maize crop⁷

Isolates	100% RDK	75% RDK	50% RDK
Control	122.96 ± 2.19 ^g	112.27 ± 1.23 ^g	103.82 ± 0.92 ^f
OPVS-1	122.77 ± 0.91 ^g	121.17 ± 2.07 ^f	114.13 ± 1.19 ^e
OPVS-2	132.94 ± 1.73 ^{ef}	124.81 ± 1.81 ^{ef}	115.88 ± 0.87 ^e
OPVS-3	135.54 ± 0.84 ^{def}	126.95 ± 0.94 ^{de}	120.47 ± 1.16 ^d
OPVS-4	137.65 ± 0.95 ^{cde}	134.28 ± 1.14 ^{bc}	124.52 ± 1.76 ^c
OPVS-5	133.34 ± 1.47 ^{ef}	128.32 ± 0.67 ^{de}	122.52 ± 0.74 ^{cd}
OPVS-6	139.77 ± 1.29 ^{cd}	135.73 ± 2.99 ^{bc}	132.94 ± 0.84 ^b
OPVS-7	140.98 ± 1.79 ^c	137.25 ± 2.09 ^b	131.65 ± 0.31 ^b
OPVS-8	141.43 ± 0.50 ^c	126.41 ± 2.48 ^{def}	122.30 ± 1.07 ^{cd}
OPVS-9	130.92 ± 1.86 ^f	123.48 ± 1.38 ^{ef}	122.45 ± 1.10 ^{cd}
OPVS-10	156.01 ± 3.41 ^a	145.42 ± 0.51 ^a	137.41 ± 1.74 ^a
OPVS-11	147.25 ± 0.58 ^b	143.07 ± 1.11 ^a	132.72 ± 0.81 ^b
OPVS-12	133.01 ± 0.80 ^{ef}	131.07 ± 2.12 ^{cd}	121.87 ± 0.71 ^{cd}

Interaction effects of all doses of potassium application with various KSB strains. OPVS-10 KSB strains with application of 75% RDK recorded ~ 6% higher plant height compared 50% RDK. The application of 100% RDK was recorded ~ 7 and 13% higher plant height compared 75 and 50% RDK, respectively. On an average of all three RDK's, the KSB strains can be arranged in decreasing order of plant

⁷ Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

height as OPVS-10 > OPVS-11 > OPVS-7 > OPVS-6 > OPVS-4 > OPVS-8 > OPVS-12 > OPVS-5 > OPVS-3 > OPVS-9 > OPVS-2 > OPVS-1 and control pot (Fig. 4.18). Earlier researchers also have showed that KSB promote plant growth (Lin *et al.*, 2002; Egamberdiyeva and Hoflich, 2003; Basak and Biswas, 2009).

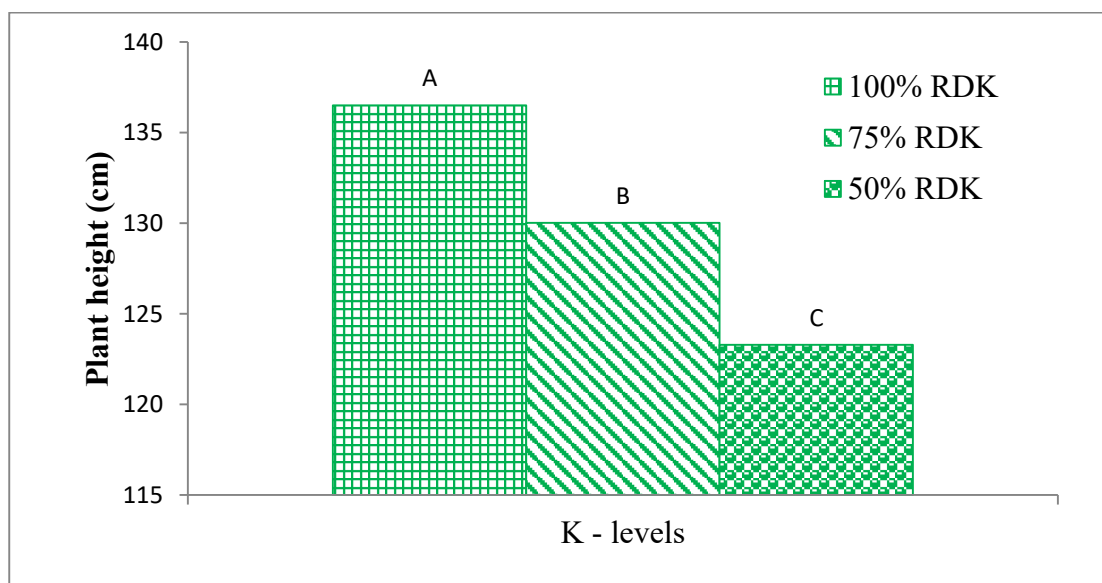


Fig. 4.18: Interaction effects of KSB and levels of potassium fertilization on plant height at 60 DAS of maize crop under net house condition. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

All the 12 KSB strains promoted the growth of maize plants to different degrees. OPVS-10 with all three doses of potassic fertilization had the most pronounced ability to promote plant growth (Table 4.16 and Fig. 4.19). This was for two reasons. First, KSB promotes the growth of maize crop by secreting auxin, and the other several growth parameters which promote nutrient uptake. Second, KSB was found to dissolve slow-release K compounds in the soil, which increased the concentration of available K in the soil, further facilitating plant growth. This statement is confirmed to the findings of (Coroneos *et al.*, 1996; Hinsinger *et al.*, 1996). It has been shown that the degradation of slow-release K compounds, such as K-feldspar is related to the organic acids secreted by plant roots (Wang *et al.*, 2000; Moritsuka *et al.*, 2004). This effect may be due to the fact that KSB solubilize a large

amount of K from K-feldspar powder as supported by earlier research workers (Maurya *et al.*, 2014; Meena *et al.*, 2015).

4.12.2 Stem girth

The results of the net housemaize experiment showed that the stem girth of maize was positively and significantly influenced by various KSB strains and different levels of RDK application (Table 4.17). Data showed that with 100% RDK the stem girth varied from 2.63 to 6.57 cm with various KSB strains. Pot with OPVS-10 caused significantly higher (6.57 cm) stem girth which was ~ 150% higher than uninoculated pot treatment (2.63 cm). Among the treated pots, OPVS- 3 and 4 noticed significantly lowest increase (~ 17%) of stem girth as compared to control, which was ~ 53% lower from the highest (6.57 cm) with OPVS-10 KSB strain. In terms of stem girth KSB strains followed the order of OPVS-10 > OPVS-9, > OPVS-6, > OPVS-7 > OPVS-11 > OPVS-8 > OPVS-12 > OPVS-5 ≥ OPVS-2 > OPVS-3 ≥ OPVS-4 > OPVS-1 and control pot.

The application of 75% RDK noticed for significantly lower stem girth in comparison to 100% RDK. However, percent increase with KSB strains was higher. Significantly higher stem girth (5.97 cm) was recorded with OPVS-10 KSB strain treated pot and lowest value (2.07 cm) observed with control pot. Which was ~ 188% higher stem girth as compared to control and it was higher (~ 150%) in comparison to 100% RDK treated pots. Data showed a higher efficiency of KSB strains with 75% RDK as compared to 100 and 50% RDK. Among the twelve KSB strains the significantly lower stem girth (2.27 cm) was observed with OPVS-4 which was ~ 62% lower as compared to KSB strain OPVS-10 (Table 4.17).

Similar to above, 50% RDK recorded lower stem girth among the all three levels of potassium. However, variation among the inoculated pot and control pot was more with 50% RDK than 100 and 75% RDK. Results showed that the significantly higher (5.00 cm) stem girth was recorded with OPVS-10. It was ~ 173, 146% higher as compared to 100% RDK in case of control pot and OPVS-3 KSB strain treated pot, respectively. It was significantly superior to rest of the KSB strains, followed by KSB strain OPVS-7 (4.70 cm) stem girth. The strains were arranged in decreasing order of

stem girth as followed OPVS-10 > OPVS-7 > OPVS-9 > OPVS-8 > OPVS-12 > OPVS-6 > OPVS-11 > OPVS-2 > OPVS-1 > OPVS-5 > OPVS-4> OPVS-3 and control pots.

Table 4.18: Effect of different KSB and levels of potassium on stem girth (cm) of maize⁸

Isolates	100% RDK	75% RDK	50% RDK
Control	2.63 ± 0.09 ^h	2.07 ± 0.07 ^f	1.83 ± 0.03 ^f
OPVS-1	3.10 ± 0.06 ^g	2.70 ± 0.12 ^{ef}	2.50 ± 0.06 ^e
OPVS-2	4.10 ± 0.06 ^f	3.43 ± 0.20 ^d	2.77 ± 0.15 ^e
OPVS-3	3.07 ± 0.07 ^g	2.33 ± 0.09 ^{ef}	2.03 ± 0.03 ^f
OPVS-4	3.07 ± 0.09 ^g	2.27 ± 0.09 ^{ef}	2.07 ± 0.09 ^f
OPVS-5	4.10 ± 0.06 ^f	2.77 ± 0.47 ^e	2.13 ± 0.07 ^f
OPVS-6	5.73 ± 0.12 ^{bc}	4.77 ± 0.15 ^{bc}	3.77 ± 0.15 ^d
OPVS-7	5.53 ± 0.20 ^{cd}	4.77 ± 0.29 ^{bc}	4.70 ± 0.10 ^b
OPVS-8	5.17 ± 0.12 ^{de}	4.73 ± 0.22 ^{bc}	4.10 ± 0.06 ^c
OPVS-9	6.03 ± 0.09 ^b	5.37 ± 0.22 ^b	4.60 ± 0.21 ^b
OPVS-10	6.57 ± 0.12 ^a	5.97 ± 0.09 ^a	5.00 ± 0.12 ^a
OPVS-11	5.20 ± 0.31 ^{de}	4.17 ± 0.09 ^c	3.67 ± 0.03 ^d
OPVS-12	5.10 ± 0.06 ^e	4.47 ± 0.15 ^c	3.97 ± 0.03 ^{cd}

data on interaction effects of KSB strains with different levels of K fertilization showed that the increase in level of K fertilization from 50 to 75%, the KSB strain OPVS- 10 gave ~ 19% higher stem girth (Table 4.17). However, another additional dose of 25% RDK (from 75 to 100%) the percent increase in stem girth recorded lower, which has ~ 10% increase over control. Data showed that the efficiency of KSB strains was reduced with application of higher doses of K

⁸ **Interaction effects were not significant**, Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P <0.05) level of significance according to DMRT.

fertilization. However, ~ 31% higher stem girth was recorded with OPVS- 10 receiving 100 RDK as compared to 50% RDK. This observation is in agreement with the findings of Babatola (2006) who reported that increase in levels of fertilizer application increase the growth and yield of crops. An optimum plant height is claimed to be positively correlated with productivity of plant (Saeed *et al.*, 2001). Stem girth of harvested maize crop treated with KSB strains, being higher and longer as compared to those from untreated plants.

4.12.3 Chlorophyll content

Results showed that the effects of various KSB strains and K fertilization significantly enhanced chlorophyll content of maize crop under net house condition (Table 4.19). Data showed that significantly higher (46.30) chlorophyll content was recorded with application of 100% RDK and OPVS-9 KSB strain which was significantly superior over the rest of the KSB strains except OPVS-4, 10 and 11. OPVS-9 KSB strain produced ~ 68 higher chlorophyll content as compared to control (27.48) pot, however, the OPVS-1 KSB strain showed ~ 25% lower chlorophyll content as compared to OPVS-9 strain. Significantly higher chlorophyll content was recorded with OPVS-9 followed by OPVS-11 (45.32), which was ~ 65% compared to control pot.

Application of 75% RDK with OPVS-4 strain gave significantly higher chlorophyll content 42.26. Which was ~ 91% higher as compared to control. Similarly, all KSB strains showed higher efficiency with 75% RDK. In terms of chlorophyll content KSB strains follow the order of OPVS-4 > OPVS-9 > OPVS-11 > OPVS-7 > OPVS-10 > OPVS-12 > OPVS-6 > OPVS-3 > OPVS-5 > OPVS-8 > OPVS-2 > OPVS-1 (Table 4.18).

Inoculation of various KSB strains with 75 and 100% RDK caused ~ 67 and 68% significantly greater chlorophyll content in comparison to 50% RDK. Significantly higher (35.03) chlorophyll content was recorded with OPVS-4, which was ~ 19% higher as compared to OPVS-11 KSB strain followed by the OPVS-8 KSB strain (34.29). It was significantly superior to rest of the KSB strains except OPVS- 8, 9 and 12. Data showed that all KSB strains followed the order OPVS-4 >

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OPVS-8 > OPVS-12 > OPVS-9 > OPVS-3 > OPVS-5 > OPVS-2 > OPVS-10 > OPVS-1 > OPVS-7 > OPVS-6 > OPVS-11 and control pots. Similar results also reported by several researchers (Khalid *et al.*, 2006; Bulgarelli *et al.*, 2013).

Table 4.18: Effect of different KSB strains and levels of potassium on chlorophyll content (SPAD value) of maize

Isolates	100% RDK	75% RDK	50% RDK
Control	27.48 ± 0.67 ^g	22.14 ± 0.63 ^h	20.97 ± 0.74 ^e
OPVS-1	34.63 ± 0.31 ^f	33.39 ± 0.69 ^g	31.32 ± 0.52 ^{bcd}
OPVS-2	37.03 ± 0.26 ^e	34.14 ± 0.67 ^{fg}	31.94 ± 0.82 ^{bc}
OPVS-3	38.53 ± 0.61 ^{de}	36.55 ± 1.09 ^{de}	32.04 ± 0.41 ^{bc}
OPVS-4	45.24 ± 0.52 ^a	42.26 ± 0.59 ^a	35.03 ± 0.82 ^a
OPVS-5	39.56 ± 0.39 ^{cd}	35.87 ± 0.44 ^{ef}	31.75 ± 0.65 ^{bc}
OPVS-6	40.98 ± 0.83 ^c	37.58 ± 0.52 ^{cde}	30.40 ± 1.03 ^{cd}
OPVS-7	40.47 ± 0.40 ^{cd}	38.95 ± 1.23 ^{bc}	30.91 ± 0.89 ^{bcd}
OPVS-8	40.14 ± 1.07 ^{cd}	35.56 ± 0.54 ^{ef}	34.29 ± 0.72 ^a
OPVS-9	46.30 ± 0.62 ^a	41.22 ± 0.61 ^a	32.95 ± 0.38 ^{ab}
OPVS-10	44.67 ± 0.88 ^{ab}	38.61 ± 0.52 ^{bcd}	31.42 ± 0.37 ^{bcd}
OPVS-11	45.32 ± 0.68 ^a	40.44 ± 0.43 ^{ab}	29.35 ± 0.49 ^d
OPVS-12	43.02 ± 0.24 ^b	38.60 ± 0.53 ^{bcd}	32.97 ± 0.74 ^{ab}

Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P <0.05) level of significance according to DMRT.

Interaction of K fertilization with various KSB strains significantly influenced on the chlorophyll content in maize leave. Data showed that the increase in doses of K fertilization with various KSB strains from 50 to 75% RDK caused ~ 21% higher value (Fig. 4.20). Further additional of 25% RDK (from 75 to 100% RDK), increase in chlorophyll content was recorded as ~ 9%. These results showed that the efficiency of KSB strains was retarded with application of higher doses of potassium. However, the ~ 32% higher chlorophyll content was recorded with pot receiving 100% RDK as

compared to 50% RDK. The increase in chlorophyll content may be attributed to supply of fertilizers and biological fixation of nutrients (Kumar *et al.*, 2014; Maurya *et al.*, 2015).

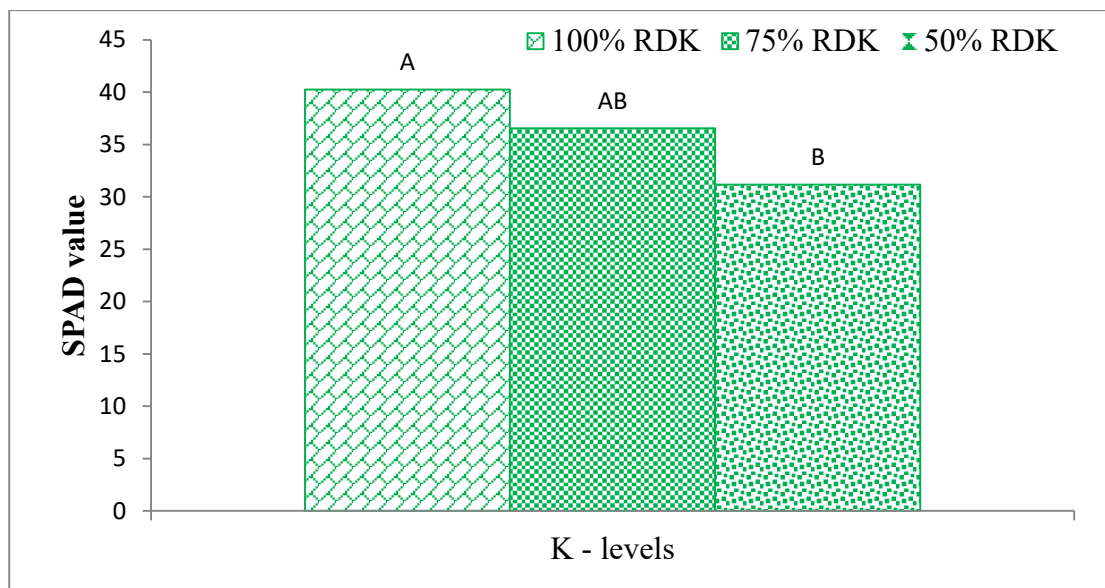


Fig. 4.20: Interaction effect of various KSB strains and levels of K fertilization on chlorophyll content (SPAD value) at 60 DAS of maize crop.

4.12.4 Number of leaves per plant

Data presented in Fig. 4.21 showed that the number of leaves per maize plant significantly influenced by the various KSB strains with levels of K fertilization. The application of 100% RDK along with various KSB strain showed that the number of leaves per plant varied from 7.67 to 16.33. Significantly higher (16.33) leaves per plant was recorded with KSB strain OPVS-10. It was ~ 59% higher as compared to control pot. The OPVS-10 showed its significant superiority over the rest of the KSB strain. With the application of 75% RDK with OPVS-10 strain produced significantly higher leaves per plant (14.67). Which was ~ 75% higher than control. The OPVS-10 showed its significant superiority over the rest of the KSB strains. Application of 50% RDK was showed for relatively lower number of leaves per plant as compared to 100 and 75% RDK. Highest number of leaves per plant was recorded with OPVS-11 followed by OPVS-12 and 10. The OPVS-11 showed its significant superiority over the rest of the KSB strains, except OPVS-12 and 10. The application of 75% RDK

showed ~ 6% higher leaves than 50% RDK. However, in case of 100% RDK, ~ 9% higher leaves were recorded as compared to 75% RDK. On an average all three doses of RDK and the KSB strains can be arranged in decreasing order of leaves per plant as OPVS-10 > OPVS-11 > OPVS-12 > OPVS-8 > OPVS-7 > OPVS-9 > OPVS-6 > OPVS-5 > OPVS-3 > OPVS-4 > OPVS-2 > OPVS-1 and control pot (El-Husseini *et al.*, 2012; Iqbal *et al.*, 2012).

4.12.5 Cob length of maize

Results of net house maize experiment showed that the cob length of maize was significantly influenced by various KSB strains along with different levels of RDK application (Table 4.20). Data showed that with 100% RDK, the cob length varied from 12.18 to 14.94 cm with various KSB strains. Pot with OPVS-10 produces ~ 21% higher length of cob as compared to uninoculated control (12.18 cm). Among the treated pots, OPVS- 3 and 4 gave ~ 8% greater but significantly shorter cob length as compared to control. In terms of cob length KSB strains followed the order of OPVS-10 > OPVS-5 > OPVS-8 > OPVS-6 > OPVS-12 > OPVS-2 > OPVS-7 > OPVS-9 > OPVS-11 > OPVS-1 > OPVS-3 ≥ OPVS-4.

Significantly higher cob length (14.78 cm) was recorded with OPVS-6 strain with 75% RDK. It was ~ 26% higher cob length as compared to control pot. The higher value of cob length was recorded with OPVS-5, 6, 8, 9, 10, 11 which differed significantly from KSB strains OPVS-1, 2, 3, 4, 7, 12. By KSB strain caused longer cob length with 75% RDK as compared to 100 and 50% RDK. Among the twelve KSB strains, OPVS-1 showed lowest value of cob length (12.79 cm), which was ~ 13% lower than OPVS-6 (Table 4.19). Similarly the application of various KSB strains along with 75 and 100% RDK was recorded for producing longer cob length compared to 50% RDK. Significantly higher (13.03 cm) cob length was recorded with OPVS-8 which was ~ 15% higher than OPVS-2 KSB strain followed by the OPVS-5 (12.97 cm) and it was at par to the rest of the KSB strains except OPVS- 1 and 2. Data showed the results of all KSB strains followed the order of OPVS-8 > OPVS-5 > OPVS-10 > OPVS-4 > OPVS-12 > OPVS-7 > OPVS-3 > OPVS-6 > OPVS-11 > OPVS-9 > OPVS-1 > OPVS-2 and control.

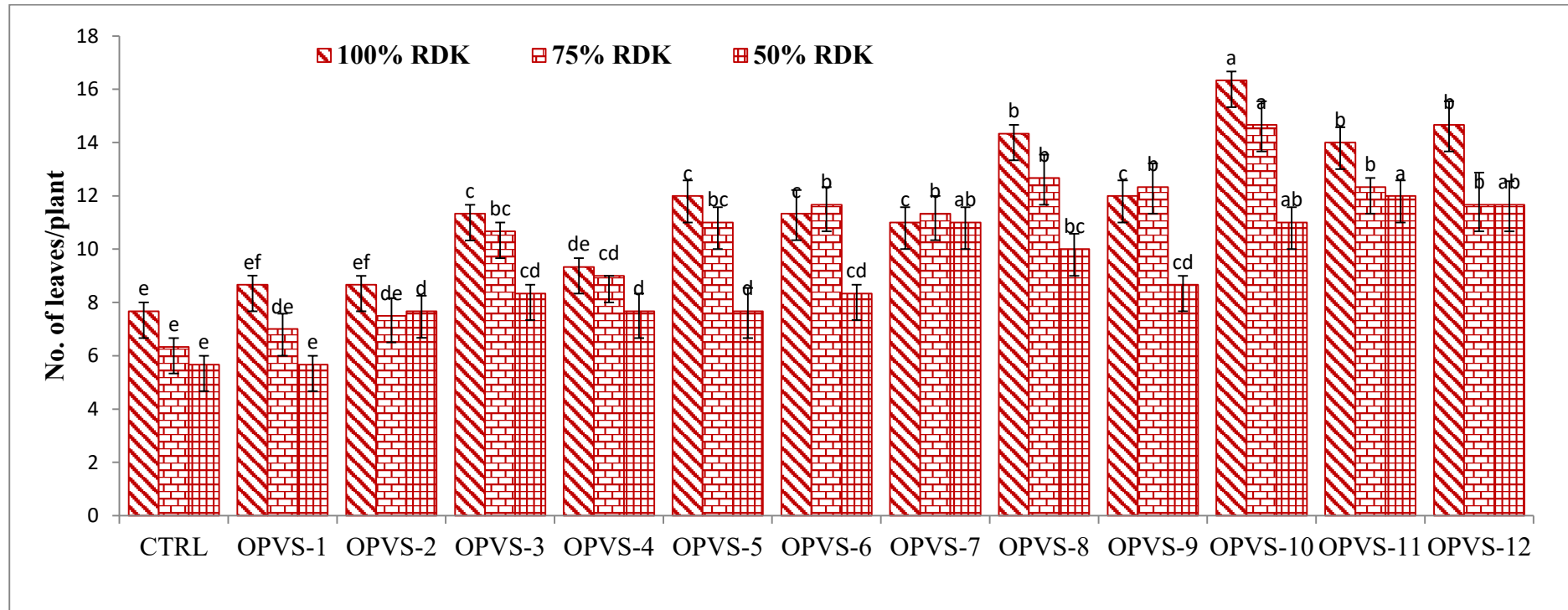


Fig. 4.20: Effect of potassium solubilizing bacteria (KSB) and levels of potassium on number of leaves/plant of maize crop under net house condition⁹. Data are presented as mean $\pm$ standard error (n = 3), Mean followed by similar letter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

⁹Interaction effects were not significant

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Effects of K fertilization without KSB also gave marked effect on cob length, but relatively lower than with KSB strains in compared to control, it was noticed that application of 100% RDK, which was ~ 4% higher cob length over 75% RDK, it was ~ 15% higher than 50% RDK. From these results it was concluded that first increment give higher responses compare to second. The data of maximum value with different KSB strains showed that the increasing K levels from 50 to 75% RDK increased ~ 15% cob length. Additional 25% dose of RDK (from 75 to 100% RDK) the percent increased in cob length was ~ 4% lower cob length of maize (Table 4.19).

Table 4.19: Effect of different KSB strains and levels of K fertilization on cob length of maize crop under net house condition¹⁰

Isolates	Cob length (cm)		
	100% RDK	75% RDK	50% RDK
Control	11.18 ± 0.41 ^e	11.75 ± 0.35 ^d	10.21 ± 0.15 ^d
OPVS-1	13.52 ± 0.28 ^d	12.79 ± 0.36 ^{cd}	11.80 ± 0.28 ^{bc}
OPVS-2	13.68 ± 0.31 ^{bcd}	13.36 ± 0.32 ^{bc}	11.33 ± 0.30 ^c
OPVS-3	13.40 ± 0.10 ^d	13.14 ± 0.42 ^c	12.35 ± 0.24 ^{abc}
OPVS-4	13.15 ± 0.12 ^d	13.02 ± 0.13 ^c	12.84 ± 0.38 ^{ab}
OPVS-5	14.64 ± 0.20 ^{ab}	14.59 ± 0.31 ^{ab}	12.97 ± 0.32 ^a
OPVS-6	14.36 ± 0.25 ^{abc}	14.78 ± 0.75 ^a	12.15 ± 0.36 ^{abc}
OPVS-7	13.67 ± 0.30 ^{bcd}	13.30 ± 0.52 ^{bc}	12.58 ± 0.38 ^{ab}
OPVS-8	14.43 ± 0.42 ^{abc}	14.58 ± 0.36 ^{ab}	13.03 ± 0.38 ^a
OPVS-9	13.64 ± 0.31 ^{bcd}	13.90 ± 0.46 ^{abc}	12.03 ± 0.33 ^{abc}
OPVS-10	14.94 ± 0.43 ^a	14.76 ± 0.28 ^a	12.92 ± 0.21 ^a
OPVS-11	13.63 ± 0.25 ^{bcd}	13.80 ± 0.26 ^{abc}	12.10 ± 0.40 ^{abc}
OPVS-12	14.04 ± 0.44 ^{abcd}	13.40 ± 0.26 ^{bc}	12.77 ± 0.34 ^{ab}

Data are presented as mean ± standard error (n = 3), Mean followed by similar letter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

¹⁰Interaction effects were not significant

Results showed that efficiency of KSB strains reduced with an increase in levels of K fertilization. However, ~ 19% higher cob length was recorded with pot receiving 100% RDK as compared to 50% RDK. Results of our study showed that inoculation of KSB along with different levels of K fertilization enhanced maize growth and yield under net house condition. This may be attributed to the enhanced nutrient uptake due to the proliferated roots through growth promoting activity of PGPR through different plant growth promoting activities, mineral solubilization along with some other mechanisms (Singh *et al.*, 2010; Ahmad *et al.*, 2012). This may also be due to improvement in soil chemical properties and nutritional status, and increased microbial activity in the root zone due to the integrated effects of KSB strains with K fertilization (Baldi *et al.*, 2010; Zhang and Kong, 2014).

4.13 Effect of KSB strains and levels of K fertilization on yield attributes of maize under net house condition

4.13.1 Grains per cob

Data on grains per cob of maize showed that, it was significantly influenced by the different KSB strains along with K fertilization (Table 4.20). With application of 100% RDK along with various KSB strain showed that the grains per cob varied from 208.33 to 276.33, it was significantly ~ 33 and ~ 25% higher grains per cob was recorded with KSB OPVS-10 as compared to OPVS-6 and uninoculated pot, respectively. The OPVS-10 showed its significantly superiority over rest of the KSB stains. The grains per cob of maize influenced by the KSB isolates was recorded in the following order OPVS-10 > OPVS-4 > OPVS-11 > OPVS-7 OPVS-3 OPVS-9 ≥ OPVS-12 > OPVS-8 > OPVS-2 > OPVS-5 > control pot > OPVS-1 and OPVS-6.

Significantly higher grains per cob (245.33) were recorded with OPVS-10 along with 75% RDK treated pot. It was ~ 27 and ~ 26% higher as compared to control and OPVS-1, respectively. This treatment combination was significantly superior to the rest of the KSB strains, except OPVS- 4, 7 and 11. In terms of grains per cob KSB strains followed the order of OPVS-10 > OPVS-11 > OPVS-4 > OPVS-7 > OPVS-3 > OPVS-8 > OPVS-12 > OPVS-9, OPVS-2 > OPVS-5 > OPVS-6 > OPVS-1 and control. However, in case of 50% RDK relatively lower grains per cob

as compared to higher levels of RDK was observed. The highest grains per cob (235) were noticed with KSB OPVS-10 strain that was ~ 26% higher than control pot (186). The OPVS-10 KSB strain showed its significant superiority over the rest of the KSB strains, except OPVS-11. Followed by OPVS-10, strain OPVS-11 gave 228.33 grains per cob of maize, which was ~ 23% higher than control pot (Table 4.21).

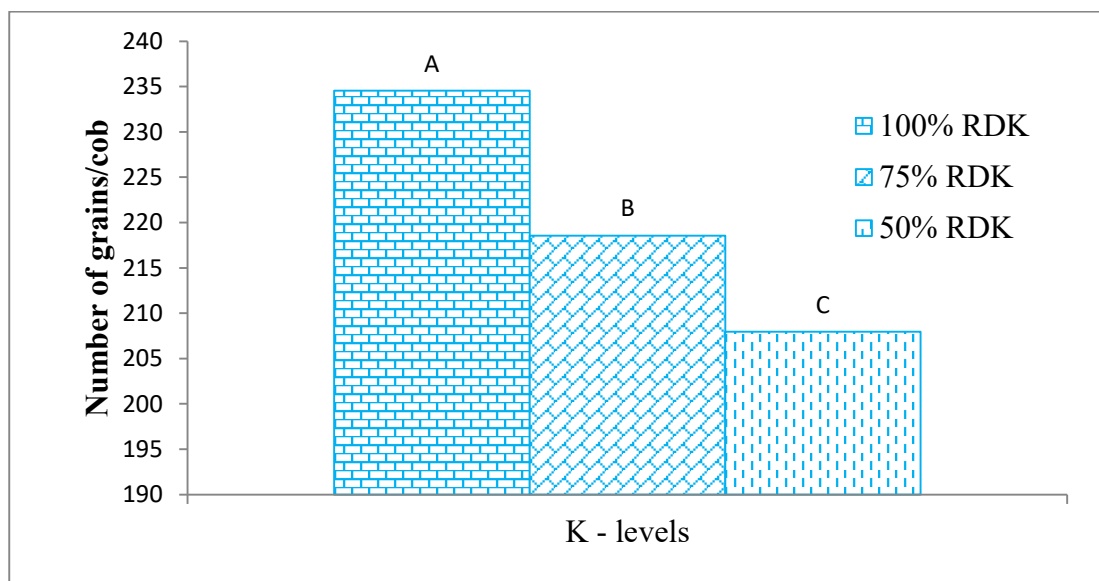


Fig. 4.21: Interaction effects of different KSB strains and levels of K fertilization on number of grains/cob of maize crop.

Effect of different K fertilization without KSB strains also showed noticeable difference. The application of 75% RDK showed ~ 4% higher grains/cob than 50% RDK. However, in case of 100% RDK ~ 19% higher grains/cob was recorded as compared to 75% RDK. The OPVS-10 along with application of 75% RDK gave ~ 5% higher grains per cob as compared to 50% RDK. The application of 100% RDK was recorded ~ 13 and 18% higher grains per cob as compared to 75 and 50% RDK, respectively (Fig. 4.22). On an average all three RDK's, the KSB strains can be arranged in decreasing order of grains per cob as OPVS-10 > OPVS-11 > OPVS-4 > OPVS-3 > OPVS-7 > OPVS-8 ≥ OPVS-12 > OPVS-9 > OPVS-5 > OPVS-2 > OPVS-1 > OPVS-6 and control. KSB along with fertilization enhanced the organic nitrogen, readily available nutrients, hormones, humic acids and vitamins etc. (Mikled *et al.*,

1994; Liu *et al.*, 2012) which might enhanced plant growth and yield through enhanced nutrient availability and improved soil health.

4.13.2 100-grain weight

Data pertaining to 100-grains weight of maize (Table 4.20) significantly influenced with various KSB strain and different levels of K fertilization treated pots as compared to control pot. However, the rate of increase in 100-grain weight differed with the various potassium solubilizing bacterial (KSB) strains. The KSB strains brought out a significant and marked effect on 100-grain weight, compared to control. Application of 100% RDK gave significantly higher 100-grain weight (~ 30 g) of maize crop as recorded with OPVS-10 KSB strain and it showed significant superiority to rest of the KSB strains. The OPVS-10 KSB strain produced ~ 53% higher 100-grain weight as compared to uninoculated control (19.64 g) and ~ 36% as compared to OPVS-1 (22.02 g) treated pot, followed by the above, strain OPVS-5 gave 28.81 g 100-grain weight that was ~ 47% greater than control pot.

The pot receiving 75% RDK along with various KSB strains showed significantly higher (26.44 g) 100-grain weight with OPVS-10 strain treated pot. It was ~ 39% higher grain weight as compared to control and showed its significant superiority over the rest of the KSB strains and control pot, followed by the OPVS-12 (24.55 g) in terms of 100-grain weight. The strains were arranged in decreasing order for increasing the 100-grain weight they were followed the order of OPVS-10 > OPVS-12 > OPVS-11 > OPVS-7 > OPVS-8 > OPVS-4 > OPVS-9 > OPVS-5 > OPVS-3 > OPVS-6 > OPVS-2 > OPVS-1 and control pots (Table 4.20).

Application of 50% RDK along with KSB strains caused relatively lower 100-grain weight as compared 100 and 75% RDK. Significantly higher grain weight (~ 24 g) was recorded with OPVS-12 KSB strain that was ~ 27% higher than control pot (~ 19 g). The OPVS-12 showed its significantly superiority over the rest of the KSB strains except OPVS-7, 9, 10 and 11.

Interaction effects of K fertilization with KSB strains caused very less increase in 100-grain weight, but significantly higher grain weight was recorded with 100% RDK followed by 75 and 50% RDK. Significantly higher value with KSB strains with application of 75% RDK gave ~ 11% higher grain weight as compared to 50% RDK. The application of 100% RDK was noticed ~ 13 and 26% higher 100-grain weight as compared to 75 and 50% RDK, respectively (Fig. 4.20). It may also be due to the production of amino acids, vitamins and growth promoting substances like indole acetic acid and gibberellic acid by these introduced beneficial microorganisms which resulted in enhanced nutrient uptake, translocation and synthesis of photosynthate assimilates and resulted increase in plant growth characters and economically profitable yield (Singh *et al.*, 2006; Suke *et al.*, 2011).

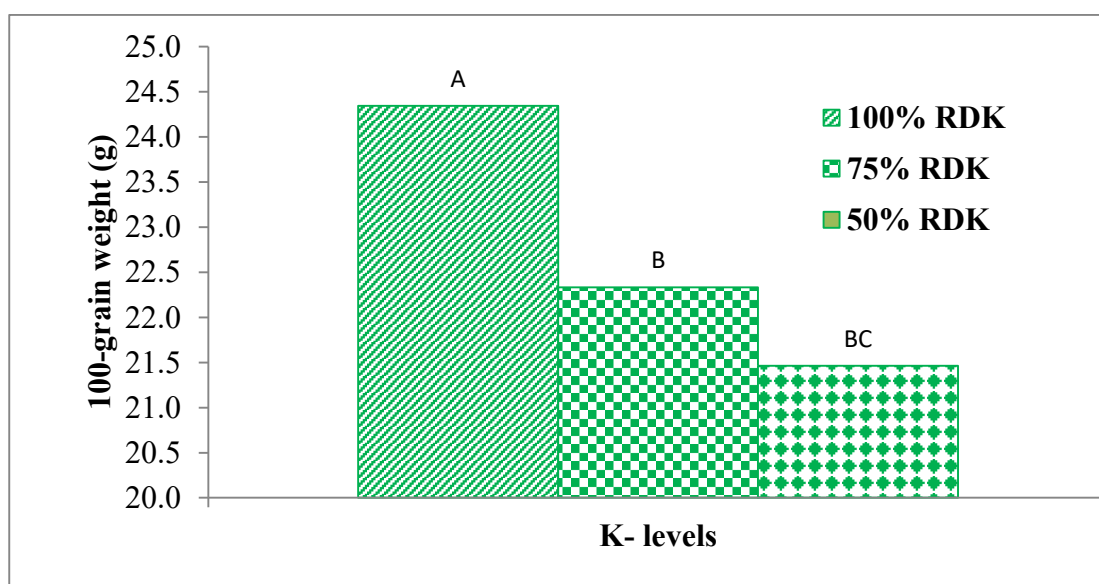


Fig. 4.22: Interaction effects of KSB and levels of K-fertilization on 100-grain weight of maize.

4.13.3 Stover yield

The KSB strains with different levels of RDK application positively and significantly enhanced stover yield of maize under net house condition (Table 4.21). Data showed that 100% RDK with various KSB strains, the stover yield varied from 175.91 to 277.72 g/plant.

Table 4.20: Effect of different KSB strains and levels of K fertilization on number of grain/cob and 100-grain weight of maize¹¹

Isolates	Number of grains/cob			100- grain weight (g)		
	100% RDK	75% RDK	50% RDK	100% RDK	75% RDK	50% RDK
Control	221.33 ± 1.77 ^d	193.33 ± 1.77 ^g	186.00 ± 3.06 ^g	19.64 ± 0.29 ^f	19.00 ± 0.45 ^a	18.69 ± 0.45 ^d
OPVS-1	219.00 ± 0.58 ^d	194.33 ± 2.34 ^g	191.00 ± 2.08 ^{fg}	22.02 ± 0.49 ^e	20.91 ± 0.40 ^e	21.42 ± 0.36 ^{bc}
OPVS-2	222.33 ± 2.03 ^d	211.00 ± 1.53 ^{de}	195.33 ± 2.61 ^{fg}	22.64 ± 0.36 ^{de}	21.39 ± 0.32 ^{de}	21.04 ± 0.61 ^{bc}
OPVS-3	240.67 ± 1.20 ^c	227.00 ± 5.30 ^{bc}	214.67 ± 3.72 ^{cd}	22.84 ± 0.24 ^{de}	21.50 ± 0.29 ^{de}	20.37 ± 0.39 ^c
OPVS-4	264.67 ± 0.67 ^b	236.33 ± 3.93 ^a	223.00 ± 2.00 ^{bc}	22.59 ± 0.11 ^{de}	22.57 ± 0.77 ^{cd}	20.92 ± 0.60 ^{bc}
OPVS-5	221.67 ± 4.81 ^d	205.67 ± 2.97 ^{ef}	202.67 ± 3.72 ^{ef}	28.81 ± 0.45 ^b	21.80 ± 0.32 ^{de}	20.26 ± 0.52 ^{cd}
OPVS-6	208.33 ± 4.41 ^e	198.00 ± 1.00 ^{fg}	196.67 ± 5.55 ^{fg}	23.75 ± 0.45 ^{cd}	21.04 ± 0.48 ^e	20.28 ± 0.71 ^{cd}
OPVS-7	244.00 ± 2.08 ^c	235.00 ± 4.51 ^{ab}	215.67 ± 3.39 ^{cd}	23.68 ± 0.30 ^{cd}	22.83 ± 0.40 ^{cd}	22.39 ± 0.49 ^{ab}
OPVS-8	223.67 ± 3.85 ^d	219.33 ± 1.45 ^{bcd}	209.33 ± 4.81 ^{de}	24.40 ± 0.48 ^c	22.70 ± 0.28 ^{cd}	21.27 ± 0.55 ^{bc}
OPVS-9	225.00 ± 3.47 ^d	215.00 ± 3.06 ^{de}	196.00 ± 3.06 ^{fg}	24.65 ± 0.46 ^c	22.04 ± 0.42 ^{de}	22.29 ± 0.62 ^{ab}
OPVS-10	276.33 ± 3.85 ^a	245.33 ± 6.13 ^a	235.00 ± 3.61 ^a	29.99 ± 0.12 ^a	26.44 ± 0.60 ^a	23.08 ± 0.44 ^a
OPVS-11	257.00 ± 4.73 ^b	243.33 ± 2.34 ^a	228.33 ± 2.19 ^{ab}	27.95 ± 0.48 ^b	23.55 ± 0.30 ^{bc}	23.20 ± 0.62 ^a
OPVS-12	225.00 ± 4.59 ^d	217.67 ± 5.79 ^{cd}	209.67 ± 5.90 ^{de}	23.51 ± 0.53 ^{cd}	24.55 ± 0.45 ^b	23.80 ± 0.18 ^a

¹¹ Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

The pot treated with OPVS-5 produced significantly higher (~ 278 g/plant) stover yield, it was ~ 49% higher than the uninoculated control. While OPVS-3 produced significantly lower (~ 176 g/plant) stover yield as compared to control (~ 187 g/plant). The stover yield due to KSB strains followed the order of OPVS-5 > OPVS-12 > OPVS-10 > OPVS-11 > OPVS-7 > OPVS-9 > OPVS-8 > OPVS-4 > OPVS-6 > OPVS-1 > control > OPVS-2 and OPVS-3.

Application of 75% RDK gave relatively higher stover yield as compared to 100% RDK. The significantly higher stover yield (~ 286 g/plant) was recorded with OPVS-5 treated pot. It was ~ 66% higher stover yield as compared to control pot and ~ 90% higher than OPVS-2. By comparing maximum value of stover yield, KSB strain showed the higher efficiency with 75% RDK as compared to 100% RDK.

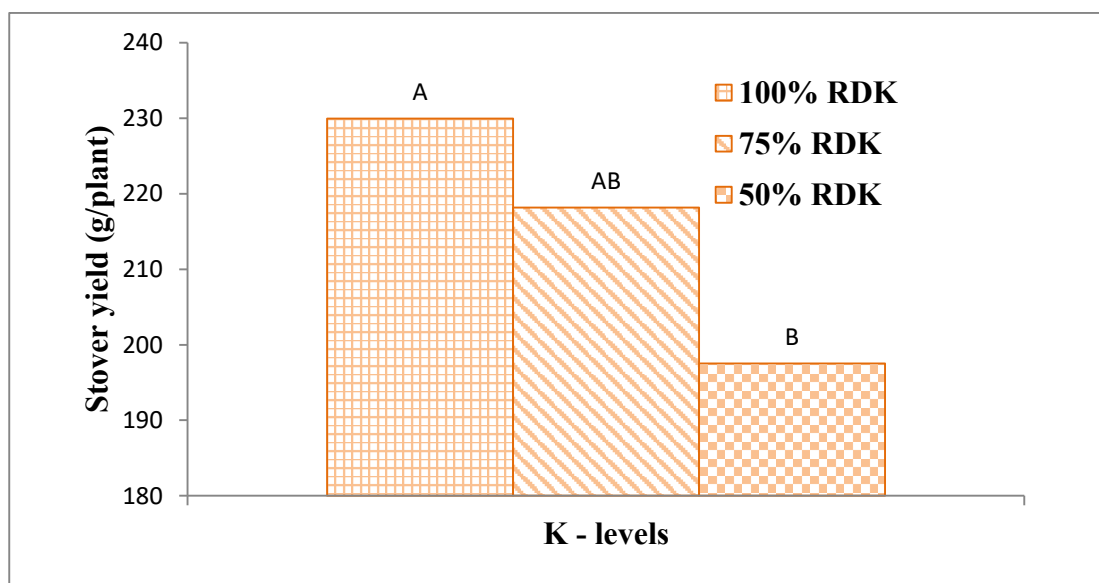


Fig. 4.23: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on stover yield of maize crop. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

Application of various KSB strains along with 50% RDK gave lower stover yield than with higher levels of K fertilization, but in terms of percent increase, it was higher from 75% RDK. It was compared to a maximum value of stover yield with 50% RDK with higher levels, it was slightly lower than 100 and 75% RDK. The

significantly higher (~ 260 g/plant) stover yield was recorded with OPVS-10, which was ~ 93% higher as compared to KSB strain OPVS-2 followed by the OPVS-5 (243.41 g/plant). The OPVS-10 showed its significantly superiority over the rest of the KSB strains. Data showed that KSB strains followed the order of OPVS-10 > OPVS-5 > OPVS-12 > OPVS-11 > OPVS-6 > OPVS-7 > OPVS-8 > OPVS-9 > OPVS-4 > OPVS-1 > control > OPVS-3 and OPVS-2 in producing the stover yield (Table 4.23). Application of 100% RDK gave ~ 9% greater stover yield as compared to 75% and the 75% RDK gave ~ 13% more stover yield than 50% RDK.

Data on stover yield showed that the increase in K levels from 50 to 75% RDK increased ~ 10% stover yield. In another additional dose, 25% RDK (from 75 to 100% RDK) the negative impact on stover yield was recorded. These results showed the efficiency of KSB strains was reduced with increase in levels of potassium application. However, the ~ 7% higher stover yield was recorded with pot receiving 100% RDK as compared to 50% RDK (Fig. 4.24). Potassium is a primary plant nutrient that plays a major role in achieving the maximum economic yields. The response to K application was significant to both grain and stover, implying that higher levels of K might have increased yields further. This increase in yield is in accordance with the essential requirements for K for photosynthesis, water relationships, protein synthesis in enzyme systems within the plant (Baldi *et al.*, 2010; Singh *et al.*, 2010; Zhang and Kong, 2014).

4.13.4 Grain yield

It is apparent from the data presented in Table 4.21 that inoculation with KSB strains significantly influenced grain yield of maize. However, the rate of increment varied with the various KSB strains. Results showed that the grain yield significantly influenced by the different KSB strains along with all three K levels (100, 75 and 50% RDK) under net house conditions.

Significantly higher grain yield (~ 170 g/plant) of maize was recorded with application of 100% RDK + OPVS-10 KSB strain, which was significantly superior to rest of the KSB strains. This strain (OPVS-10) produced ~ 53% higher grain yield

as compared to control. The OPVS-2 KSB strain showed ~ 25% lower yield as compared to OPVS-10. The strain OPVS-11 was recorded at second which yielded ~ 158 g/plant which was ~ 42% higher than the control (Table 4.21 and Fig. 4.25).

With 75% RDK with OPVS-10 gave maximum grain yield ~ 150 g/plant which was ~ 39% significantly higher as compared to control. The OPVS-10 showed its significant superiority over the rest of the KSB strains. The grain yield of maize influenced by various KSB strains along with levels of K fertilization followed the order of OPVS-10 > OPVS-11 > OPVS-12 > OPVS-9 > OPVS-5 > OPVS-8 > OPVS-7 > OPVS-4 > OPVS-1 > OPVS-3 > OPVS-2 > OPVS-6.

Application of 50% RDK caused lowest grain yield among all three levels of potassium. The maximum value (~ 135 g/plant) was recorded with OPVS-12. It was ~ 27% significantly higher than control (~ 106 g/plant). This isolate showed its significant superiority over the rest of the KSB strains except OPVS- 5, 7, 9, 10 and 11.

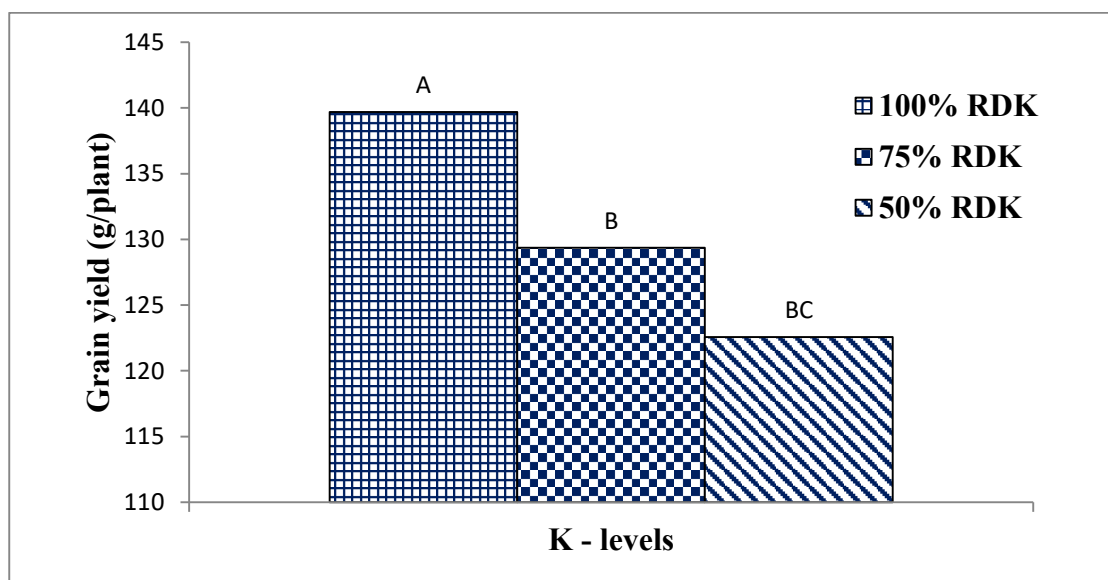


Fig. 4.24: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on grain yield of maize crop.

The KSB strain OPVS-11 ranked at seconds, which caused significantly higher grain yield (~ 132 g/plant). The strains can be arranged in decreasing order of

grain yield as followed OPVS-12 > OPVS-11 > OPVS-10 > OPVS-8 > OPVS-9 > OPVS-5 > OPVS-1 > OPVS-8 > OPVS-2 > OPVS-4 > OPVS-3 > OPVS-6 (Table 4.24). Increase the potassium from 50 to 75% increased ~ 11% of grain yield and further increase to 100% RDK caused ~ 13% increase in grain yield of maize (Fig. 4.25). Potassium has this impact because it is involved in over 60 enzyme systems that regulate plant growth reactions. One of these major roles is in regulating water use efficiency in the plant. The opening and closing of the stomates in the leaves is directly related to the concentration of potassium in the cells that surround the stomates. Carbohydrates provide energy for plant growth when they are broken down which potassium plays a key role in grain yield. Many reports have shown a contribution to the growth of maize plants by plant growth promoting bacteria. Some studies showed that the extent of positive bacterial effects on plant growth may vary between the species or on different genotypes of the same crop (Mitter *et al.*, 2013; Zuniga *et al.*, 2013; Arruda *et al.*, 2013).

4.14 Effect of KSB strains and levels of K fertilization on maize rhizospheric nutrient availability (NPK) under net house condition

4.14.1 Available nitrogen

Data on available nitrogen at harvest of maize crop presented in table 4.24 showed that the available nitrogen significantly influenced by the different levels of K fertilization and various KSB strains under net house condition. At 100% RDK significantly higher (116.88 mg/kg) available N with OPVS-5, followed by OPVS-10, 8, 11 and 9 with ~116, 114, 114, 113 mg/kg was recorded no much variation among the KSB strain was observed.

Application of 75% RDK along with KSB strain OPVS-8 gave significantly higher (115.73 mg/kg) available N content in soil after harvest of the maize crop, followed by OPVS-5 and 10 KSB strains. This was significantly superior to rest of the KSB strains along with 75% RDK and it was ~13% higher as compared to control (~100 mg/kg) pot. With 50% RDK and OPVS-10 showed significantly higher (~ 106 mg/kg) available N which was at par with OPVS-2 and 5 strains of KSB. The OPVS-10 gave ~ 13% higher value of available N as compared to control (~ 92 mg/kg).

Table 4.21: Effect of different KSB strain and levels of K fertilization on stover and grain yields of maize¹²

Isolates	Stover yield (g/plant)			Grain yield (g/plant)		
	100% RDK	75% RDK	50% RDK	100% RDK	75% RDK	50% RDK
Control	186.86 ± 1.75 ^f	171.75 ± 1.86 ^g	152.67 ± 3.82 ^f	111.34 ± 1.67 ^g	107.73 ± 2.56 ^g	105.99 ± 2.56 ^e
OPVS-1	201.04 ± 2.84 ^e	189.91 ± 2.31 ^f	177.61 ± 1.79 ^e	128.70 ± 1.47 ^f	122.23 ± 1.40 ^{ef}	121.47 ± 2.06 ^{bcd}
OPVS-2	183.89 ± 5.40 ^f	150.25 ± 0.55 ^h	134.26 ± 5.03 ^g	128.37 ± 2.05 ^f	121.26 ± 1.79 ^{ef}	119.30 ± 3.48 ^{bcd}
OPVS-3	175.91 ± 3.35 ^f	178.51 ± 1.84 ^g	142.26 ± 4.92 ^{fg}	129.52 ± 1.33 ^{ef}	121.91 ± 1.64 ^{ef}	115.48 ± 2.19 ^{de}
OPVS-4	229.95 ± 6.32 ^d	241.99 ± 2.78 ^c	179.11 ± 2.61 ^e	136.96 ± 1.35 ^d	127.95 ± 4.35 ^{def}	118.59 ± 3.43 ^{cd}
OPVS-5	277.72 ± 6.14 ^a	285.90 ± 2.28 ^a	243.41 ± 1.69 ^b	154.62 ± 1.46 ^b	134.65 ± 1.18 ^{cd}	126.24 ± 8.86 ^{abcd}
OPVS-6	229.21 ± 3.16 ^d	232.86 ± 3.60 ^d	221.50 ± 1.61 ^c	134.69 ± 2.53 ^{de}	119.28 ± 2.72 ^f	115.01 ± 4.03 ^{de}
OPVS-7	252.93 ± 3.61 ^c	208.91 ± 2.44 ^e	204.84 ± 2.83 ^d	140.56 ± 2.02 ^{cd}	129.43 ± 2.29 ^d	126.95 ± 2.77 ^{abcd}
OPVS-8	230.28 ± 4.03 ^d	214.97 ± 3.53 ^e	198.00 ± 2.64 ^d	138.35 ± 2.73 ^d	132.64 ± 0.80 ^{cd}	120.58 ± 3.14 ^{bcd}
OPVS-9	231.29 ± 1.15 ^d	192.45 ± 1.33 ^f	195.98 ± 4.13 ^d	139.75 ± 2.62 ^{cd}	133.46 ± 1.09 ^{cd}	126.38 ± 3.50 ^{abcd}
OPVS-10	260.64 ± 2.50 ^{bc}	252.30 ± 1.87 ^b	259.91 ± 6.42 ^a	170.04 ± 0.68 ^a	149.91 ± 3.38 ^a	130.86 ± 2.52 ^{abc}
OPVS-11	258.87 ± 1.89 ^{bc}	230.78 ± 3.34 ^d	221.52 ± 2.63 ^c	158.48 ± 2.70 ^b	142.03 ± 0.96 ^b	131.56 ± 3.51 ^{ab}
OPVS-12	270.61 ± 4.27 ^{ab}	285.44 ± 5.06 ^a	236.48 ± 4.90 ^b	144.66 ± 1.54 ^c	139.21 ± 2.57 ^{bc}	134.96 ± 1.05 ^a

¹² Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

This strain showed its significantly superiority over the rest of the KSB strains (Table 4.24). KSB strains along with levels of K fertilization also influenced significantly the available N after harvest of maize crop under net house condition. With 100% RDK significantly higher (~ 111 mg/kg) available N followed by 75 (~ 108 mg/kg) and 50% RDK (~ 100 mg/kg) was gave significantly ~ 3 and 11% higher available N with 75 and 50% RDK, respectively (Fig. 4.26).

Data showed that the 100% RDK caused significantly higher available nitrogen in soil followed by 75 and 50% RDK with various KSB strains. Efficient rhizosphere microorganisms are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on growth and establishment of plants (Zhang and Kong, 2014; Kumar *et al.*, 2015).

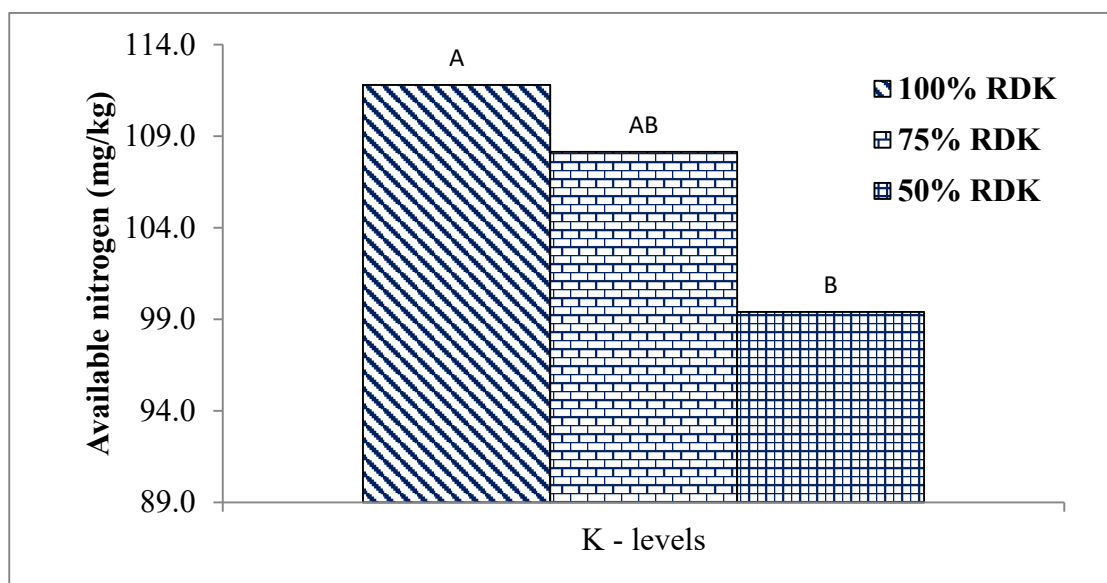


Fig. 4.25: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on available nitrogen. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.14.2 Available phosphorus

Data pertaining to available P in soil at harvest of maize crop are presented in Table 4.22. Perusal of data reveals that available P in soil was significantly higher due to inoculation of various KSB strains and levels of K fertilization after harvested soil

of maize crop under net house condition. Various KSB strains gave higher amount of available P in soil than control. Doses of K gave significantly higher values of available P with 100% RDK (~ 11 mg/kg) followed by 75% RDK (~ 10 mg/kg) and 50% RDK (~ 9 mg/kg) with various KSB strains.

Data showed that KSB strains OPVS-10 caused significantly higher (15.44 mg/kg) available P in soil with 100% RDK. However, significantly lower available P was recorded with control (6.78 mg/kg) without KSB strain, which was ~ 56 higher available P as compared control. Available P varied from 6.78 to 15.44 mg/kg with various KSB strains. The application of 75% RDK with various KSB strains varied significantly among the KSB strain which ranged from 5.83 to 13.30 mg/kg available. It was ~ 52% higher as compared to control (5.83 mg/kg) pots under net house condition. OPVS-10 KSB strains showed its significantly superiority over rest of the KSB strains with all three K fertilization levels. 100% RDK gave significantly more available P which was superior to 75 and 50% RDK.

Data showed that OPVS-10 caused ~ 12 mg/kg available P along with 50% RDK which showed its significantly superiority over the rest of the KSB strains. the ranged of available P from 5.17 to 11.89 mg/kg of available P at harvested soil of the experiment of crop. The highest value of available P was registered with the 100, 75 and 50% RDK along with OPVS-10 KSB strain (Table 4.26 and Fig. 4.26). It was significantly superior over the rest of the KSB strains. This may be due to mobilization of potassium from minerals because of secretion of organic acids by the bacterial strain, which in turn increased the biomass yield.

The significantly higher available P might be due to increase in the amount of root exudates and increased microbial activity leading to greater mineralization of applied and inherent P. Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, and Ca) and decrease the pH in basic soils (Stevenson, 2005).

Table 4.22: Effect of different KSB strains and levels of K fertilization on available N and P rhizospheric soil of maize¹³

Isolates	Available nitrogen (mg/kg)			Available Phosphorus (mg/kg)		
	100% RDK	75% RDK	50% RDK	100% RDK	75% RDK	50% RDK
Control	102.82 ± 0.69 ^c	100.78 ± 1.85 ^f	92.26 ± 1.26 ^e	6.78 ± 0.29 ^f	5.83 ± 0.44 ^f	5.17 ± 0.17 ^e
OPVS-1	110.72 ± 0.96 ^b	110.46 ± 1.33 ^{bc}	103.69 ± 1.01 ^{ab}	9.52 ± 0.38 ^e	8.80 ± 0.36 ^e	7.68 ± 0.43 ^d
OPVS-2	110.02 ± 0.90 ^b	105.88 ± 0.86 ^{cde}	101.04 ± 1.40 ^{abc}	11.21 ± 0.55 ^{cd}	9.43 ± 0.23 ^{de}	8.61 ± 0.31 ^d
OPVS-3	110.18 ± 0.65 ^b	108.16 ± 2.61 ^{cd}	100.03 ± 2.34 ^{bc}	9.95 ± 0.26 ^{de}	9.35 ± 0.40 ^{de}	8.71 ± 0.47 ^d
OPVS-4	109.30 ± 1.58 ^b	103.15 ± 1.26 ^{ef}	96.01 ± 1.68 ^{cde}	11.94 ± 0.16 ^{bc}	10.61 ± 0.26 ^{bcd}	8.82 ± 0.40 ^{cd}
OPVS-5	116.88 ± 1.27 ^a	114.08 ± 0.83 ^{ab}	104.55 ± 1.47 ^{ab}	10.68 ± 0.29 ^{cde}	9.79 ± 0.24 ^{de}	9.10 ± 0.32 ^{cd}
OPVS-6	110.02 ± 0.90 ^b	107.61 ± 0.62 ^{cd}	97.11 ± 1.05 ^{cde}	11.43 ± 0.42 ^c	9.96 ± 0.30 ^{cde}	9.02 ± 0.49 ^{cd}
OPVS-7	109.47 ± 1.68 ^b	102.53 ± 1.67 ^{ef}	93.51 ± 1.52 ^{de}	12.83 ± 0.30 ^b	11.55 ± 0.37 ^b	10.98 ± 0.64 ^{ab}
OPVS-8	114.68 ± 0.61 ^a	115.73 ± 1.96 ^a	99.50 ± 1.12 ^{bc}	9.99 ± 0.41 ^{de}	9.49 ± 0.16 ^{de}	8.90 ± 0.42 ^{cd}
OPVS-9	113.98 ± 0.80 ^a	107.05 ± 1.76 ^{cde}	100.38 ± 2.00 ^{bc}	10.07 ± 0.65 ^{de}	9.46 ± 0.43 ^{de}	9.06 ± 0.37 ^{cd}
OPVS-10	116.56 ± 1.04 ^a	113.65 ± 1.71 ^{ab}	105.98 ± 1.14 ^a	15.44 ± 0.66 ^a	13.30 ± 0.79 ^a	11.89 ± 0.19 ^a
OPVS-11	114.13 ± 1.44 ^a	106.95 ± 1.33 ^{cde}	98.01 ± 1.93 ^{cd}	11.90 ± 0.06 ^{bc}	11.24 ± 0.29 ^b	10.68 ± 0.36 ^{ab}
OPVS-12	114.69 ± 0.91 ^a	109.92 ± 1.03 ^{bc}	100.02 ± 2.52 ^{bc}	12.83 ± 0.20 ^b	11.15 ± 0.47 ^{bc}	10.38 ± 1.04 ^{bc}

¹³Data are presented as mean ± standard error (n = 3), Mean followed by similar letter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

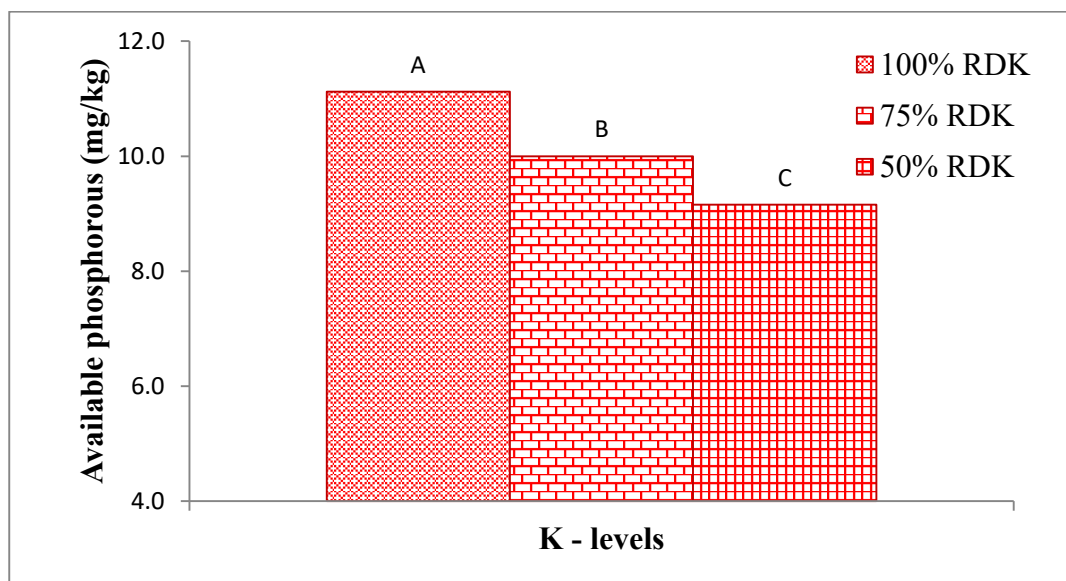


Fig. 4.26: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on available phosphorus. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.14.3 Available potassium

Data showed that content of available potassium in harvested soil trended to significant increase by various KSB strains and different levels of K fertilization (Table 4.23). 100% RDK gave significantly higher (119.43 mg/kg) amount of available K in soil with OPVS-10 followed by OPVS-11, these strains was at par with OPVS-4 (116.35 mg/kg) and OPVS-8 (112.92 mg/kg). Significantly lowest amount of available K was found in control where no KSB strain was added compared to other treatments receiving various KSB strain inoculation OPVS-10 showed its superiority over uninoculated control (98.88 mg/kg) pot (Table 4.23).

At 75% RDK, significantly greater (96.47 mg/kg) amount of available K was obtained with application of OPVS-10 and OPVS-4 (96.63 mg/kg). OPVS-11 ranked in the third and in term of percentage it gave ~13% higher available of K as compared to control pot (84.15 mg/kg).

At 50% RDK, significantly higher (75.58 mg/kg) amount of available K was recorded with OPVS-10 KSB strains, it was ~14% higher as compared to control

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(65.55 mg/kg) pot. Thereafter, it was at par with OPVS-11 (73.82 mg/kg). 100% RDK resulted significantly higher ~ 18 and 36% on 75 and 50% RDK, respectively. Treatments receiving 100% RDK along with OPVS-100 KSB strain enhanced availability of K in harvested soil of maize crop. Data presented in Fig. 4.27 showed that after 100% RDK, availability of K decreased significantly with 75 and 50% RDK. It has been shown that the degradation of slow-release K compounds, such as K-feldspar, is related to the organic acids secreted by plant roots (Moritsuka *et al.*, 2004; Maurya *et al.*, 2014; Meena *et al.*, 2015; Kumar *et al.*, 2015).

Table 4.23: Effect of potassium solubilizing bacteria (KSB) and levels of potassium on available potassium of soil after harvest of maize crop

Isolates	Available potassium (mg/kg)		
	100% RDK	75% RDK	50% RDK
Control	98.88 ± 1.30 ^e	84.15 ± 0.66 ^g	65.55 ± 0.58 ^f
OPVS-1	109.75 ± 1.17 ^{bcd}	93.13 ± 2.25 ^{bc}	75.58 ± 0.64 ^a
OPVS-2	103.70 ± 1.13 ^{de}	86.06 ± 0.29 ^{efg}	71.53 ± 0.57 ^{bc}
OPVS-3	104.02 ± 0.94 ^{de}	88.32 ± 0.70 ^{def}	69.80 ± 0.89 ^{cde}
OPVS-4	116.35 ± 2.01 ^{ab}	96.63 ± 1.01 ^a	75.55 ± 0.61 ^a
OPVS-5	106.92 ± 2.66 ^{cd}	88.05 ± 1.06 ^{def}	67.95 ± 0.68 ^{def}
OPVS-6	108.88 ± 3.79 ^{cd}	85.52 ± 0.52 ^{fg}	67.97 ± 0.79 ^{def}
OPVS-7	110.23 ± 1.81 ^{bcd}	92.92 ± 0.88 ^{bc}	70.27 ± 0.76 ^{cd}
OPVS-8	112.92 ± 1.95 ^{abc}	90.55 ± 0.56 ^{cd}	70.28 ± 0.67 ^{cd}
OPVS-9	108.69 ± 2.21 ^{cd}	88.70 ± 0.54 ^{de}	67.34 ± 0.63 ^{ef}
OPVS-10	119.43 ± 2.63 ^a	96.47 ± 0.78 ^a	75.58 ± 1.53 ^a
OPVS-11	117.66 ± 3.24 ^a	95.52 ± 1.15 ^{ab}	73.82 ± 1.34 ^{ab}
OPVS-12	107.07 ± 1.69 ^{cd}	86.99 ± 0.56 ^{efg}	65.80 ± 0.62 ^f

Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P <0.05) level of significance according to DMRT.

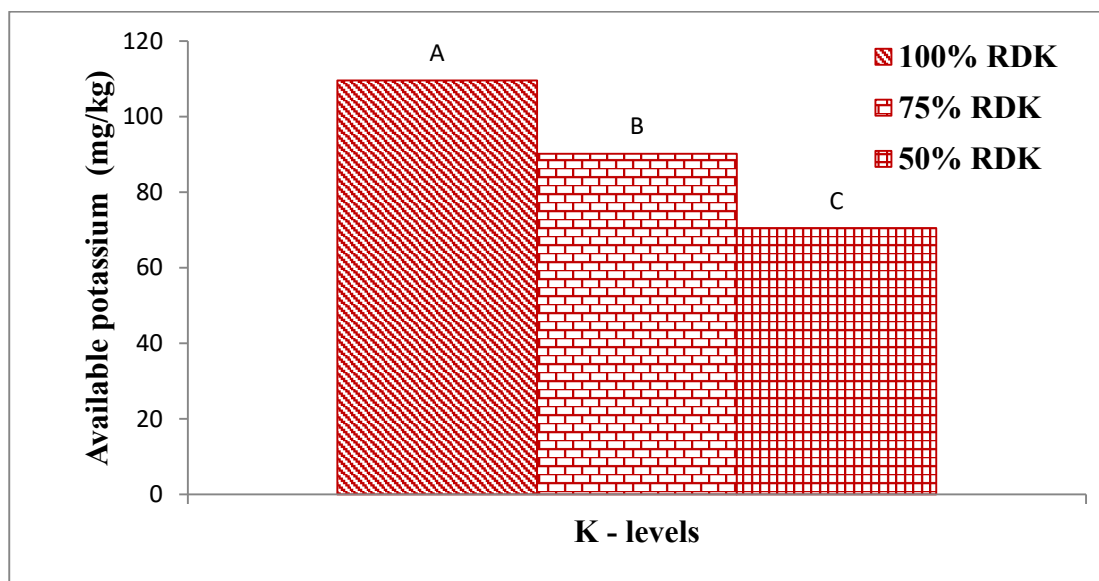


Fig. 4.27: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on available potassium. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.15 Effect of KSB strains and levels of K fertilization on nutrients uptake by maize crop

4.15.1 Nitrogen uptake by grain

Data showed that the nitrogen uptake by grain of maize was significantly influenced by various KSB strains along with different level of K fertilization (Table 4.26). 100% RDK along with OPVS-10 and 11 KSB strains treated pots showed their significant superiority over rest of the KSB strains. These isolates showed ~ 45% higher N uptake by maize grain as compared to control 1.52 g/pot. At 75% RDK, OPVS-10 and 11 KSB strains showed significantly higher 2.03 and 2.17%, respectively N uptake by grain. This was ~ 47% higher N uptake by grain compared to control pot (1.15 g/pot).

Application of 50% RDK along with KSB strain OPVS-11 (1.80 g/pot N) showed its superiority over the rest of the KSB strains. Significantly higher (2.01 g/pot) N uptake by grain with 100% RDK was recorded with 75% RDK (1.61

g/pot) and 50% RDK (1.39 g/pot). 100% RDK showed its superiority over 75 and 50% RDK. Meanwhile ~ 20 and 31% lower N uptake by grain was recorded with 75 and 50% RDK, respectively.

4.15.2 Phosphorus uptake by grain

The significantly higher (0.58 g/pot) P uptake by grain was recorded with 100% RDK followed by 75% RDK (0.47 g/pot) and 50% RDK (0.41 g/pot). Which was significantly higher ~ 19 and 30% lower P uptake by grain in comparison to with 75 and 50% RDK, respectively (Fig. 4.29).

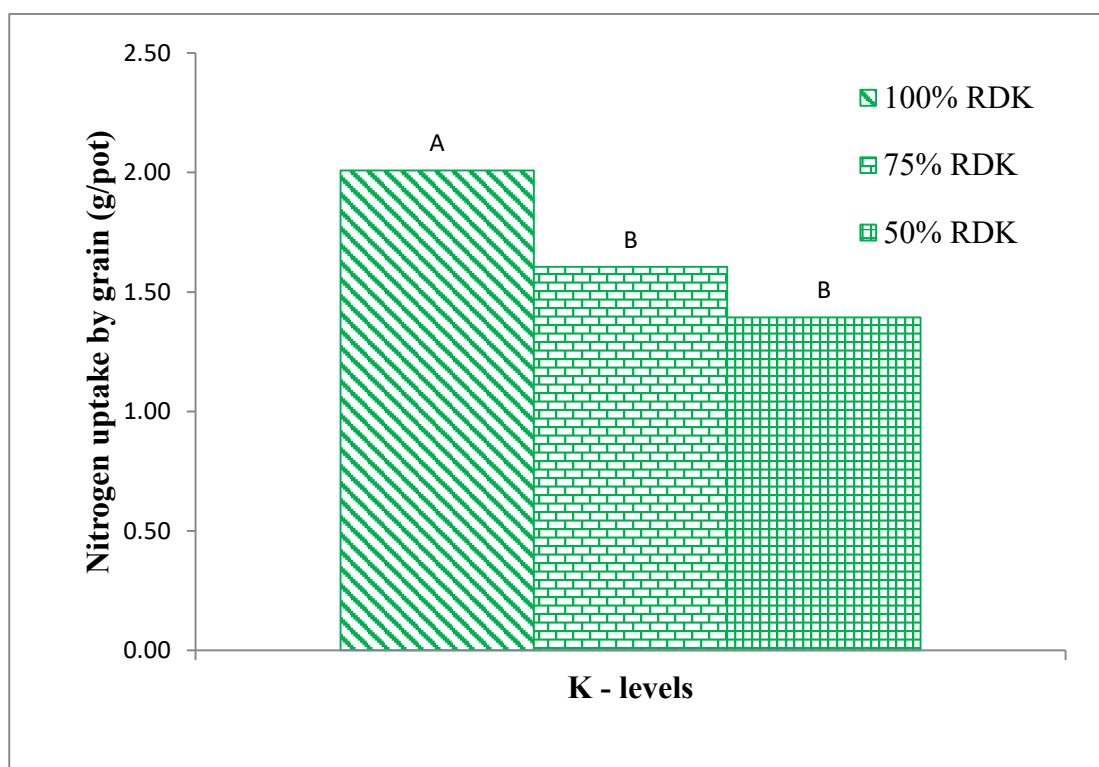


Fig. 4.28: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on nitrogen uptake by maize grain.

Data showed that the OPVS-10 gave significantly higher (0.83 g/pot) grain P uptake followed by OPVS-11 (0.73 g/pot). Which showed its significant superiority over rest of the KSB strains. Application of 75% RDK along with OPVS-10 showed significantly higher (0.63 g/pot) P uptake by maize grain (Fig. 4.29). However, in case of 50% RDK along with OPVS-10 showed its significant

superiority over the rest of the treatment, it was ~ 39% higher grain P uptake as compared to control (0.31 g/pot).

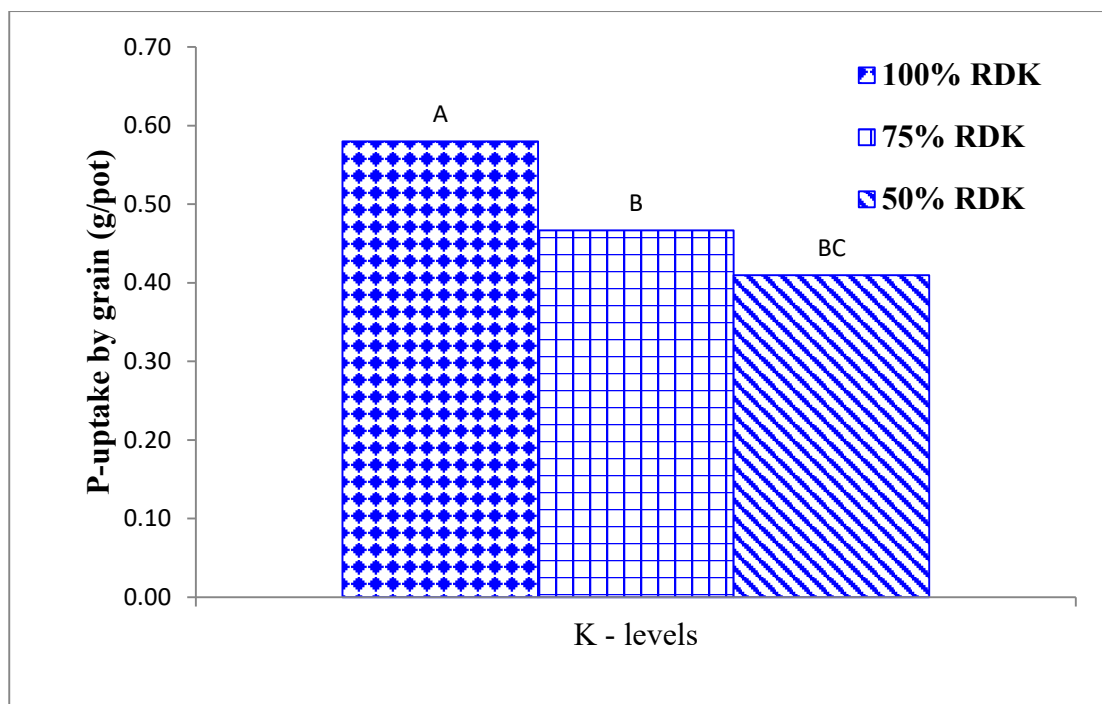


Fig. 4.29: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on P-uptake by maize grain. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.15.3 Potassium uptake by grain

Potassium uptake by grain was significantly influenced by various KSB strains and different levels of K fertilization (Table 4.25 and Fig. 4.31). With the application of 100% RDK along with various KSB strains OPVS-10 significantly higher (0.83 g/pot) K uptake by grain was recorded. Which was ~ 46% higher K uptake by grain recorded as compared to control (0.45 g/pot). 75% RDK and OPVS-10 KSB strain gave ~ 41% higher K uptake by grain as compared to control (0.40 g/pot). This strain showed it significant superiority over the rest of the KSB strains. However, in case of 50% RDK with OPVS-10 gave significantly higher (0.54 g/pot) and it was ~ 33 % higher K uptake by grain in comparasion to control.

Table 4.24: Effect of different KSB strains and levels of K fertilization on N and P uptake by maize grain¹⁴

Isolates	Nitrogen uptake by grain (g/pot)			Phosphorus uptake by grain (g/pot)		
	100% RDK	75% RDK	50% RDK	100% RDK	75% RDK	50% RDK
Control	1.52 ± 0.01 ^g	1.15 ± 0.02 ^d	0.97 ± 0.00 ⁱ	0.42 ± 0.01 ⁱ	0.33 ± 0.02 ^d	0.31 ± 0.02 ^e
OPVS-1	1.57 ± 0.01 ^f	1.21 ± 0.01 ^c	1.08 ± 0.04 ^h	0.46 ± 0.01 ^h	0.35 ± 0.02 ^d	0.34 ± 0.01 ^{cde}
OPVS-2	1.65 ± 0.05 ^f	1.37 ± 0.03 ^c	1.31 ± 0.09 ^{efg}	0.48 ± 0.00 ^{gh}	0.37 ± 0.01 ^d	0.33 ± 0.00 ^{de}
OPVS-3	1.93 ± 0.07 ^e	1.57 ± 0.05 ^b	1.27 ± 0.09 ^{fg}	0.59 ± 0.01 ^{cde}	0.47 ± 0.01 ^{bc}	0.41 ± 0.02 ^{bc}
OPVS-4	1.69 ± 0.04 ^f	1.36 ± 0.05 ^c	1.23 ± 0.04 ^{gh}	0.56 ± 0.04 ^{def}	0.48 ± 0.01 ^{bc}	0.41 ± 0.02 ^{bc}
OPVS-5	2.04 ± 0.11 ^{cde}	1.67 ± 0.03 ^b	1.40 ± 0.13 ^{defg}	0.62 ± 0.02 ^{cd}	0.48 ± 0.03 ^{bc}	0.42 ± 0.04 ^b
OPVS-6	2.13 ± 0.02 ^{bc}	1.68 ± 0.08 ^b	1.43 ± 0.04 ^{cdef}	0.60 ± 0.02 ^{cde}	0.49 ± 0.03 ^{bc}	0.43 ± 0.04 ^b
OPVS-7	2.28 ± 0.03 ^b	1.74 ± 0.04 ^b	1.56 ± 0.02 ^{bcd}	0.50 ± 0.00 ^{fgh}	0.41 ± 0.00 ^{cd}	0.39 ± 0.02 ^{bcd}
OPVS-8	2.10 ± 0.09 ^{cd}	1.64 ± 0.11 ^b	1.38 ± 0.02 ^{defg}	0.62 ± 0.03 ^{cd}	0.56 ± 0.02 ^{ab}	0.46 ± 0.02 ^{ab}
OPVS-9	2.14 ± 0.06 ^{bc}	1.74 ± 0.03 ^b	1.50 ± 0.07 ^{bcd}	0.54 ± 0.03 ^{efg}	0.49 ± 0.06 ^{bc}	0.42 ± 0.03 ^b
OPVS-10	2.78 ± 0.03 ^a	2.03 ± 0.05 ^a	1.62 ± 0.04 ^{abc}	0.83 ± 0.04 ^a	0.63 ± 0.01 ^a	0.51 ± 0.01 ^a
OPVS-11	2.73 ± 0.05 ^a	2.17 ± 0.09 ^a	1.80 ± 0.05 ^a	0.73 ± 0.01 ^b	0.53 ± 0.05 ^b	0.47 ± 0.02 ^{ab}
OPVS-12	1.95 ± 0.04 ^{de}	1.74 ± 0.07 ^b	1.65 ± 0.03 ^{ab}	0.64 ± 0.01 ^c	0.48 ± 0.04 ^{bc}	0.43 ± 0.02 ^b

¹⁴Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

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Interaction effects of KSB strains with different K fertilization levels was also significant with KSB strains 100% RDK showed its significantly superiority over the rest of the both 75 and 50% RDK. Significantly higher (0.60 g/pot) K uptake by grain was observed with 100% RDK followed by 75% RDK (0.51 g/pot) and 50% RDK (0.45 g/pot). There was ~ 10 and 12% higher K uptake by grain as compared to 75 and 50% RDK with 100% RDK > 75% RDK and 50% RDK in decreasing order (Fig. 4.30). Inoculants have been shown to influence the uptake of many other elements in addition to N and P (Peix *et al.*, 2001; Khan, 2005; Wu *et al.*, 2005; Adesemoye *et al.*, 2008). Han and Lee (2005) reported an increased uptake of P and K when soil was fertilized with rock P and K and co-inoculated with P solubilizing bacteria *B. megaterium* and K solubilizing bacteria *B. mucilaginosus*.

Table 4.25: Effect of different KSB strain and levels of K fertilization on K-uptake by maize grain¹⁵

Isolates	Potassium uptake by grain (g/pot)		
	100% RDK	75% RDK	50% RDK
Control	0.45 ± 0.00 ^h	0.40 ± 0.01 ^d	0.36 ± 0.02 ^c
OPVS-1	0.50 ± 0.02 ^g	0.43 ± 0.01 ^c	0.38 ± 0.01 ^{bc}
OPVS-2	0.53 ± 0.01 ^g	0.45 ± 0.01 ^c	0.42 ± 0.03 ^{abc}
OPVS-3	0.54 ± 0.01 ^{fg}	0.44 ± 0.01 ^c	0.41 ± 0.04 ^{abc}
OPVS-4	0.58 ± 0.01 ^{ef}	0.47 ± 0.02 ^c	0.41 ± 0.05 ^{abc}
OPVS-5	0.69 ± 0.03 ^{bc}	0.57 ± 0.01 ^b	0.49 ± 0.01 ^{ab}
OPVS-6	0.56 ± 0.01 ^{fg}	0.47 ± 0.02 ^c	0.42 ± 0.05 ^{abc}
OPVS-7	0.61 ± 0.00 ^{de}	0.54 ± 0.01 ^b	0.50 ± 0.05 ^{ab}
OPVS-8	0.62 ± 0.02 ^{de}	0.54 ± 0.01 ^b	0.47 ± 0.05 ^{abc}
OPVS-9	0.64 ± 0.03 ^{cd}	0.56 ± 0.02 ^b	0.49 ± 0.07 ^{ab}
OPVS-10	0.83 ± 0.01 ^a	0.68 ± 0.02 ^a	0.54 ± 0.04 ^a
OPVS-11	0.70 ± 0.02 ^b	0.58 ± 0.03 ^b	0.51 ± 0.04 ^{ab}
OPVS-12	0.63 ± 0.03 ^{de}	0.56 ± 0.02 ^b	0.50 ± 0.03 ^{ab}

¹⁵ Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

Sheng and He (2006) also has reported improved uptake of K through the inoculation of PGPR *B. edaphicus* strains NBT and suggested that the production of organic acids (citric, oxalic, tartaric, succinic, and α -ketogluconic) by the strain and its mutants lead to chelation of metals and mobilization of K from K-containing minerals.

4.15.4 Nitrogen uptake by stover

Results showed that the N uptake by stover of maize significantly influenced by various KSB strains along with different levels of K fertilization (Table 4.26 and Fig. 4.32). Significantly higher (2.27 g/pot) N uptake by stover was recorded with OPVS-11 along with 100% RDK, which was ~ 38% higher N uptake by stover compared to control 1.40 g/pot. OPVS-5 gave significantly higher (2.00 g/pot) N uptake by stover and showed its significantly superiority over rest of the KSB strains which was ~ 47% higher K uptake by stover compared to control (1.05 g/pot).

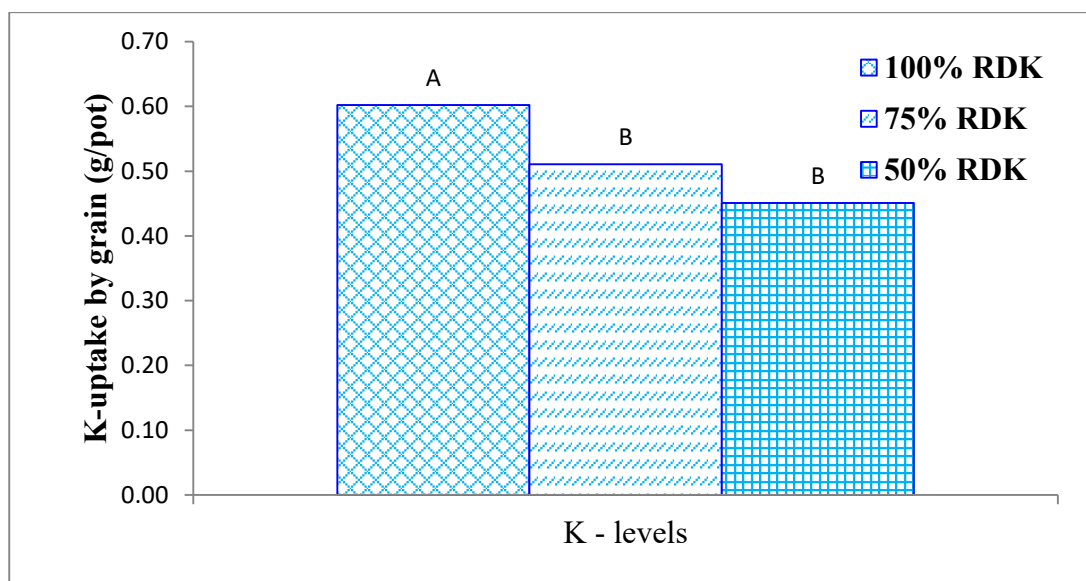


Fig. 4.30: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on K-uptake by maize grain. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

With the application of 50% RDK with various KSB strains, N uptake by stover was influenced significantly. Significantly higher N uptake (1.61 g/pot) with OPVS-10 followed by OPVS-5 (1.54 g/pot), it was ~ 42% significantly higher N uptake by stover compared to control (0.92 g/pot). Interaction effects of K fertilization and various KSB strains also significantly influenced the N uptake by stover. Significantly higher (1.67 g/pot) N uptake followed by 75% RDK (1.32 g/pot) and 50% RDK (1.13 g/pot). 100% RDK gave ~21 and 32% higher N uptake by stover as compared to 75 and 50% RDK, respectively (Fig. 4.32).

4.15.5 Phosphorus uptake by stover

Data showed that the P uptake by stover of maize influenced significantly by inoculation of various KSB strains along with levels of K fertilization. Application of 100% RDK, significantly higher (0.65 g/pot) P uptake by stover of maize was recorded with OPVS-5 and it was at par (0.64 g/pot) with OPVS-12, which showed its significantly superiority over the rest of the KSB strains. 100% RDK showed ~ 35% higher P uptake by stover of maize compared to control (0.42 g/pot) pot.

Application of 75% RDK, significantly higher (0.64 g/pot) P uptake by stover with both OPVS-5 and 12 KSB strains was recorded. Both KSB strains showed significant superiority over rest of the KSB strains. The KSB strain OPVS-5 and 12 gave ~ 41% higher P uptake by stover as compared to control (0.38 g/pot). The KSB strain OPVS-10 showed ~ 43% higher P uptake by stover with 50% RDK. This treatment was at par with OPVS-12 and 5 KSB strains and showed its significant superiority over rest of the isolates, except OPVS-12 and 5 (Table 4.32).

Interaction of various K fertilization and KSB strains showed significant variation with various KSB strains. Significantly higher (0.52 g/pot) P uptake by stover with 100% RDK, followed by 75 (0.47 g/pot) and 50% RDK (0.40 g/pot), it was ~ 10 and 23% higher with 75 and 50% RDK, respectively (Fig. 4.33).

Table 4.26: Effect of potassium solubilizing bacteria (KSB) and levels of potassium on nitrogen uptake by maize stover¹⁶

Isolates	Nitrogen uptake by stover (g/pot)			Phosphorus uptake by stover (g/pot)		
	100% RDK	75% RDK	50% RDK	100% RDK	75% RDK	50% RDK
Control	1.40 ± 0.04 ^h	1.05 ± 0.07 ^g	0.92 ± 0.08 ^{fg}	0.42 ± 0.01 ^g	0.38 ± 0.01 ^{de}	0.32 ± 0.00 ^{fg}
OPVS-1	1.42 ± 0.05 ^g	1.12 ± 0.05 ^{ef}	0.98 ± 0.12 ^{de}	0.44 ± 0.01 ^{fg}	0.40 ± 0.02 ^{cd}	0.36 ± 0.00 ^{def}
OPVS-2	1.51 ± 0.06 ^{fg}	1.03 ± 0.05 ^f	0.86 ± 0.12 ^{ef}	0.42 ± 0.00 ^g	0.32 ± 0.01 ^e	0.26 ± 0.02 ^g
OPVS-3	1.25 ± 0.04 ^h	0.86 ± 0.04 ^g	0.59 ± 0.08 ^g	0.41 ± 0.02 ^g	0.39 ± 0.01 ^{cd}	0.30 ± 0.01 ^{efg}
OPVS-4	1.23 ± 0.04 ^h	1.09 ± 0.01 ^{ef}	0.75 ± 0.03 ^{fg}	0.58 ± 0.01 ^{bcd}	0.56 ± 0.01 ^b	0.41 ± 0.02 ^d
OPVS-5	2.27 ± 0.06 ^a	2.00 ± 0.10 ^a	1.54 ± 0.02 ^a	0.65 ± 0.03 ^a	0.64 ± 0.04 ^a	0.53 ± 0.01 ^{ab}
OPVS-6	1.65 ± 0.04 ^{ef}	1.54 ± 0.05 ^{cd}	1.33 ± 0.14 ^b	0.57 ± 0.01 ^{cd}	0.55 ± 0.03 ^b	0.48 ± 0.05 ^{bc}
OPVS-7	2.07 ± 0.03 ^b	1.45 ± 0.09 ^d	1.34 ± 0.09 ^b	0.51 ± 0.02 ^c	0.38 ± 0.01 ^{cd}	0.35 ± 0.02 ^{def}
OPVS-8	1.53 ± 0.01 ^{fg}	1.20 ± 0.02 ^c	1.10 ± 0.09 ^{cd}	0.49 ± 0.04 ^{ef}	0.41 ± 0.02 ^{cd}	0.36 ± 0.01 ^{de}
OPVS-9	1.70 ± 0.01 ^{de}	1.19 ± 0.06 ^c	1.20 ± 0.06 ^{bc}	0.53 ± 0.02 ^{de}	0.42 ± 0.02 ^c	0.41 ± 0.03 ^d
OPVS-10	1.85 ± 0.13 ^{cd}	1.57 ± 0.08 ^{bcd}	1.61 ± 0.09 ^a	0.62 ± 0.02 ^{abc}	0.56 ± 0.01 ^b	0.56 ± 0.03 ^a
OPVS-11	2.27 ± 0.06 ^a	1.62 ± 0.10 ^{bc}	1.56 ± 0.07 ^a	0.53 ± 0.01 ^{de}	0.45 ± 0.02 ^c	0.42 ± 0.01 ^{cd}
OPVS-12	1.90 ± 0.01 ^c	1.67 ± 0.08 ^b	1.19 ± 0.14 ^{bc}	0.64 ± 0.02 ^{ab}	0.64 ± 0.03 ^a	0.51 ± 0.02 ^{ab}

¹⁶Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

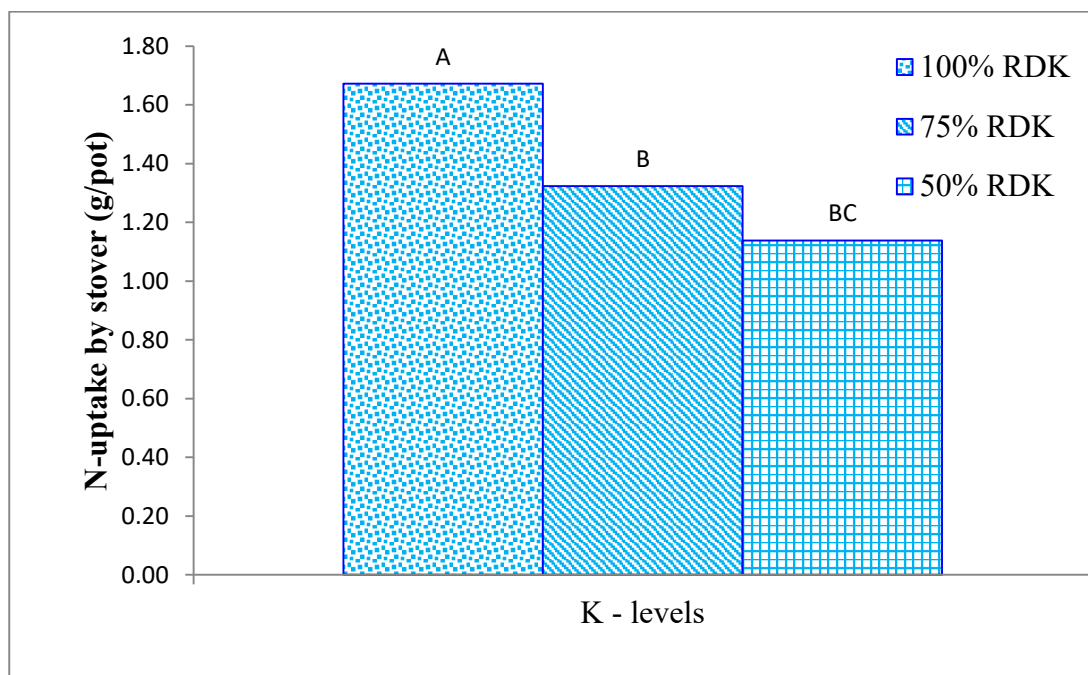


Fig. 4.31: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on N-uptake by maize stover. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

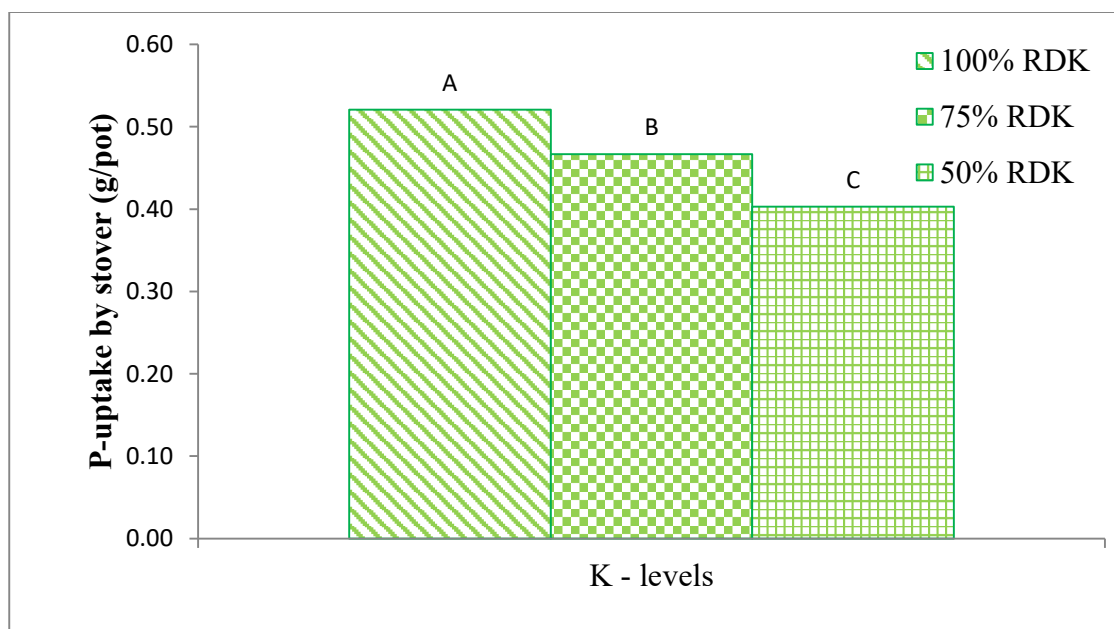


Fig. 4.32: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on P-uptake by maize stover. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.15.6 Potassium uptake by stover

Inoculation of KSB strains along with K levels significantly influenced the K uptake by stover of maize plant (Table 4.27 and Fig. 4.34). Application of 100% RDK gave significantly higher (3.92 g/pot) K uptake with 100% RDK followed by 75% RDK (3.53 g/pot) and 50% RDK (3.01 g/pot). It was ~ 10 and 23% higher K uptake as compared to 75 and 50% RDK, respectively.

Table 4.27: Effect of different KSB strains and levels of K fertilization on K-uptake by maize stover¹⁷

Isolates	Potassium uptake by stover (g/pot)		
	100% RDK	75% RDK	50% RDK
Control	3.00 ± 0.08 ^f	2.38 ± 0.05 ^h	2.04 ± 0.05 ^g
OPVS-1	3.42 ± 0.11 ^d	3.09 ± 0.08 ^f	2.76 ± 0.06 ^{ef}
OPVS-2	3.16 ± 0.15 ^{de}	2.40 ± 0.03 ^h	2.06 ± 0.07 ^g
OPVS-3	3.01 ± 0.12 ^e	2.85 ± 0.06 ^g	2.12 ± 0.07 ^g
OPVS-4	3.83 ± 0.11 ^c	3.88 ± 0.06 ^c	2.69 ± 0.05 ^f
OPVS-5	4.86 ± 0.15 ^a	4.61 ± 0.01 ^a	3.72 ± 0.22 ^{ab}
OPVS-6	3.86 ± 0.12 ^c	3.74 ± 0.08 ^c	3.36 ± 0.04 ^c
OPVS-7	4.38 ± 0.08 ^b	3.35 ± 0.06 ^{de}	3.16 ± 0.13 ^{cd}
OPVS-8	4.03 ± 0.12 ^c	3.50 ± 0.03 ^d	3.12 ± 0.08 ^{cd}
OPVS-9	3.95 ± 0.04 ^c	3.14 ± 0.08 ^{ef}	3.02 ± 0.03 ^{de}
OPVS-10	4.73 ± 0.11 ^{ab}	4.29 ± 0.11 ^b	4.14 ± 0.14 ^a
OPVS-11	4.48 ± 0.09 ^b	3.95 ± 0.14 ^c	3.30 ± 0.02 ^{cd}
OPVS-12	4.74 ± 0.15 ^{ab}	4.80 ± 0.12 ^a	3.70 ± 0.11 ^{ab}

¹⁷ Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

The OPVS-5 KSB strain showed its significant superiority over rest of the KSB strains along with levels of K fertilization. Significantly higher K uptake by stover was recorded with OPVS-5 and it was at par with OPVS-12 (4.74 g/pot) and showed OPVS-10 (4.73 g/pot) treated pots ~ 38% higher K uptake by stover compared (3.00 g/pot) control pot. The application of 75% RDK along with the KSB strains OPVS-5 and 12 gave the significantly higher 4.80 and 4.61 g/pot, respectively. It was ~ 48 higher K uptakes by stover compared to control pot (Table 4.34).

Application of 50% RDK, OPVS-10 gave the significantly higher (4.14 g/pot) K uptake by stover at par with OPVS-5 (3.72 g/pot). It showed significant superiority rest of the KSB strains, it was ~ 51 higher K uptake compared control pot (Fig. 4.34). Similarly, *Azotobacter* and *Azospirillum* inoculated with rice seedling led to increase 35–21% yield and yield component, respectively as compared to control under field condition at 0, 30 and 60 kg/ha nitrogen fertilizer. There were significant interactions between nitrogen level and biofertilizer regarding yield, yield component and protein content (Mirzaei *et al.*, 2010).

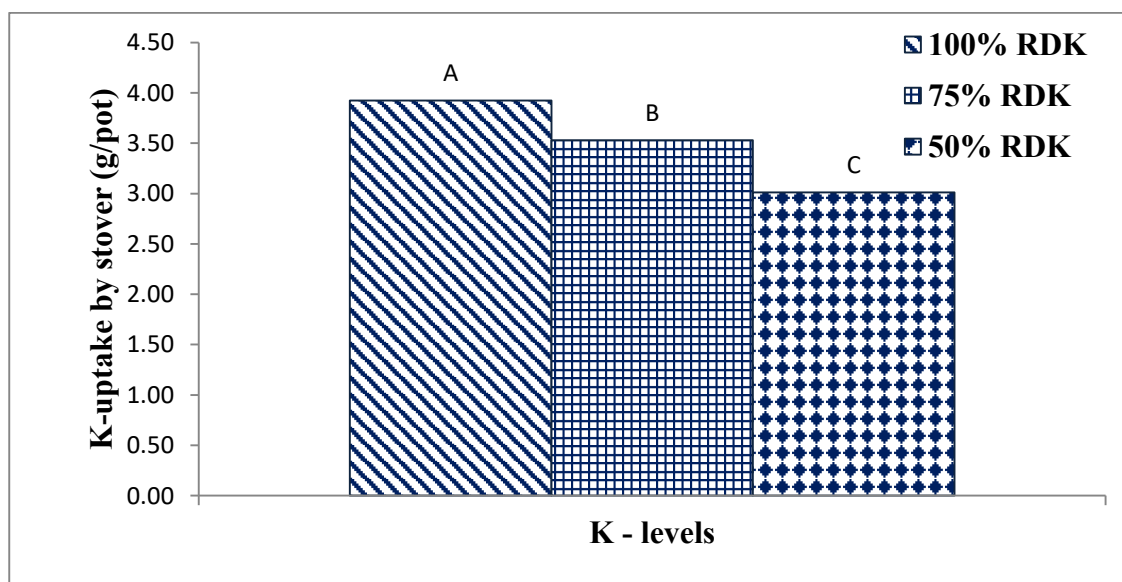


Fig. 4.33: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on K-uptake by maize stover. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

4.15.7 Total nitrogen uptake by crop

Data on total N uptake by maize crop have been presented in Table 4.34 and depicted in Figure 4.35. Data showed that the total nitrogen uptake by maize influenced positively and significantly by the inoculation of various KSB strains and graded doses of K fertilization. Data showed that the total nitrogen uptake by maize with application of 100% RDK ranged from 2.92 to 5.00 g/pot with various KSB strains. The KSB strain OPVS-11 showed significantly highest (5.00 g/pot) total nitrogen uptake which was ~ 42% higher than the uninoculated control pot (2.92 g/pot). KSB strain OPVS-11, 10 and 7 were also observed for significantly higher 4.63 and 4.34 g/pot of total N-uptake by maize crop, respectively. In terms of total N-uptake by maize KSB strains followed the order of OPVS-11 > OPVS-10 > OPVS-7 > OPVS-5 > OPVS-12 > OPVS-9 > OPVS-6 > OPVS-8 > OPVS-3 > OPVS-2 > OPVS-1 > OPVS-4 $\geq$ control (Table 4.35).

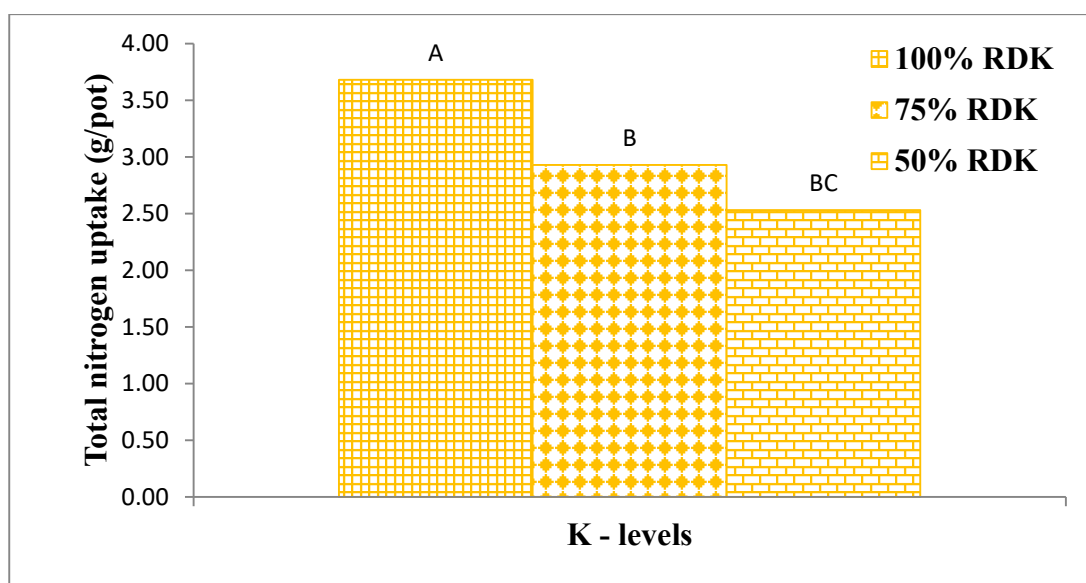


Fig. 4.34: Interaction effects of various KSB strains and levels of K fertilization on total N-uptake by crop. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

The application of 75% RDK recorded significantly decreased the total N-uptake as compared to 100% RDK. However, increase in terms of percent with various KSB strains was slightly higher. The significantly higher (3.79 g/pot) total N-uptake was recorded with OPVS-10 KSB strain treated pot and lowest total N-uptake (2.20 g/pot) observed with uninoculated control, which was ~ 41% significantly higher as compared to control and that was slightly higher as compared to 100% RDK ~ 42%. This is similar in almost all strains. It was apparent that the higher efficiency of KSB strains was with 75% RDK. Among the twelve KSB strains the lowest total N-uptake (2.33 g/pot) was observed with OPVS-1, it was ~ 39% lower as compared to OPVS-11. Among the doses of K fertilization 50% RDK caused lowest total N-uptake by maize crop. The OPVS-11 KSB strain showed ~ 43% significantly higher total N-uptake compared control pot and OPVS-3 KSB and showed significant superiority over the rest of the KSB strains except OPVS- 10 (3.23 g/pot). The strains were arranged in decreasing order of total nitrogen uptake as followed OPVS-11 > OPVS-10 > OPVS-5 > OPVS-7 > OPVS-12 > OPVS-6 > OPVS-9 > OPVS-8 > OPVS-2 > OPVS-1 > OPVS-4 > OPVS-3 (Table 4.37).

Interaction effects of various KSB strain and K fertilization levels significantly influenced total N-uptake. Among the K fertilization, increase in K level from 50 to 75%, the OPVS- 11 recorded ~ 13% significantly higher total N-uptake and with 100% RDK, which gave ~ 32% higher total N-uptake was recorded as (Fig. 4.34). However, the ~ 49% higher total N-uptake was recorded with OPVS- 11 receiving 100% RDK than 50% RDK. Such inoculation could compensate for nutrient deficiency and improve plant development through production of plant growth regulators by microbes at the root interface, which stimulate root development of plants which result in better absorption of water and nutrients from the soil (Hoflich and Kuhn, 1996; Wu *et al.*, 2005).

4.15.8 Total phosphorus uptake by crop

The application of various KSB strains with graded levels of K fertilization significantly enhanced total phosphorus uptake by maize crop (Table 4.28 and Fig. 4.36). Results showed that the application of 100% RDK with various KSB strains the

total P-uptake varied from 0.84 to 1.45 g/pot. Pot treated with OPVS-10 KSB strain gave significantly higher (1.45 g/pot) total P-uptake; it was ~ 42% significantly higher as compared to control pot. Among the all KSB strains OPVS-1, 2 and OPVS-3, 7 caused significantly lower P uptake 0.90 and 1.00 g/pot, respectively as compared to OPVS-10 KSB strain. The total P-uptake with KSB strains followed the order of OPVS-10 > OPVS-12 > OPVS-5 > OPVS-11 > OPVS-6 > OPVS-4 > OPVS-8 > OPVS-9 > OPVS-3 $\geq$ OPVS-7 > OPVS-2 $\geq$ OPVS-1 (Table 4.28).

The total P-uptake with the application of 75% RDK was relatively lower as compared to 100% RDK. The significantly higher total P-uptake (1.20 g/pot) was recorded with OPVS-10 strain treated pot, which was ~ 41% higher as compared to control and OPVS-2. It showed significant superiority over the rest of the KSB strains and at par with OPVS-12 and 5 followed by the OPVS-10, strains OPVS- 5 and 12 (1.12 g/pot), OPVS- 4 and 6 (1.04 g/pot), OPVS- 8 and 11 (0.97 g/pot) recorded relatively higher in total P-uptake from rest of the strains (Table 4.35).

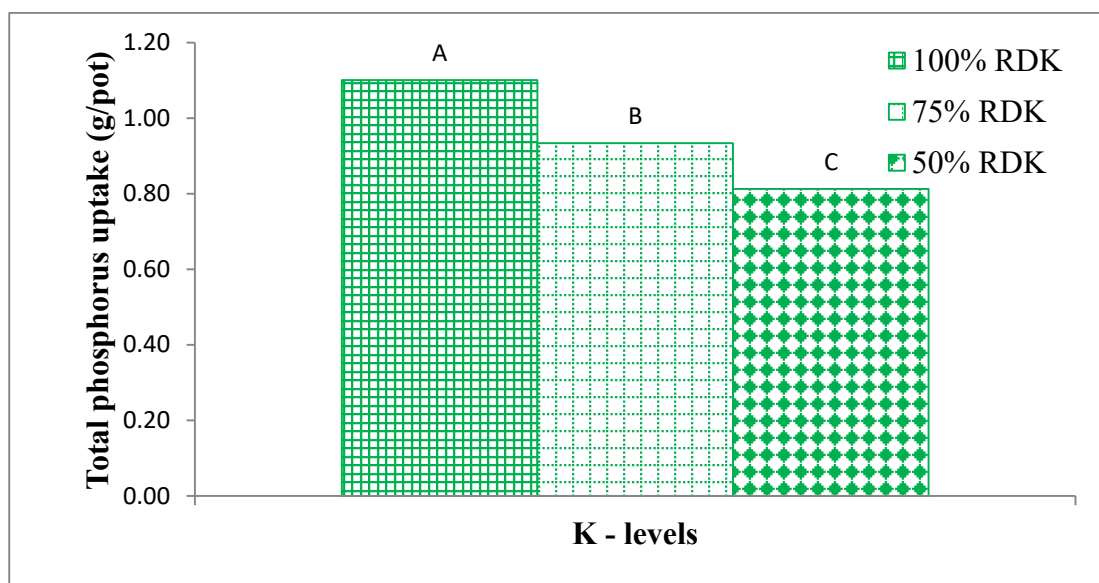


Fig. 4.35: Interaction effects of various KSB strains and levels of K fertilization on total P-uptake by crop. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to DMRT

Similarly different KSB strains along with 50% RDK gave for lower total P-uptake with the graded doses K fertilization, but in terms of per cent increase, it was

slightly higher than 75% RDK. The significantly higher (1.08 g/pot) total P-uptake was recorded with OPVS-10 which was ~ 42% higher as compared to control. OPVS-10, OPVS-5 and 12 caused 0.95 and 0.94 g/pot P uptake, respectively. The OPVS-10 showed its significant superiority over rest of the KSB strains. Data showed that all KSB strains followed the order of OPVS-10 > OPVS-5 > OPVS-12 > OPVS-6 > OPVS-11 > OPVS-8 ≥ OPVS-9 ≥ OPVS-4 > OPVS-7 > OPVS-3 > OPVS-1 and control ≥ OPVS-2 (Table 4.35).

Interaction of various KSB strains and levels of K-fertilization significantly influenced total P-uptake. However, the graded levels of K fertilization without KSB strains showed marked effect on total P-uptake but relatively lower as compared to KSB strains. In comparison to control application of 100% RDK gave ~ 18% higher than 75% and the 75% RDK gave ~ 13% significantly higher total P-uptake in compared to 50% RDK (Fig. 4.36). The uptake or accumulation of the macronutrients like N, P and K is the direct reflection of the biomass production. The result of this experiment revealed that full dose of NP along with levels of K fertilization (100, 75 and 50% RDK) with various KSB strains significantly influenced the uptake of N, P and K nutrients by crops. Wu *et al.* (2005) repeated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments.

4.15.9 Total potassium uptake by crop

Results showed that various KSB strains and levels of K fertilization significantly enhanced total K-uptake by maize crop under net house condition (Table 4.29 and Fig. 4.37). Significantly higher (5.56 g/pot) total K-uptake was recorded with the application of 100% RDK along with the OPVS-10 KSB strain, which was significantly superior to rest of the KSB strains except OPVS-5 and 12. The KSB strain OPVS-10 gave ~ 38% significantly higher total K-uptake compared to control (3.45 g/pot) pot, the KSB strains OPVS-3 gave significantly lowest (3.55 g/pot) total K-uptake which was ~ 36% significantly lower compared to OPVS-10 KSB strains. The significantly higher total K-uptake was recorded with OPVS-10 followed by OPVS-5 (5.55 g/pot).

Table 4.28: Effect of different KSB strains and levels of K fertilization on total NP-uptake by maize crop¹⁸

Isolates	Total nitrogen uptake (g/pot)			Total phosphorus uptake (g/pot)		
	100% RDK	75% RDK	50% RDK	100% RDK	75% RDK	50% RDK
Control	2.92 ± 0.04 ^h	2.20 ± 0.06 ^f	1.89 ± 0.07 ^g	0.84 ± 0.00 ^g	0.71 ± 0.01 ^f	0.63 ± 0.04 ^f
OPVS-1	2.99 ± 0.07 ^{fg}	2.33 ± 0.04 ^e	2.06 ± 0.03 ^{ef}	0.90 ± 0.01 ^f	0.74 ± 0.06 ^f	0.70 ± 0.01 ^{ef}
OPVS-2	3.16 ± 0.02 ^f	2.41 ± 0.03 ^e	2.17 ± 0.03 ^e	0.90 ± 0.01 ^f	0.69 ± 0.01 ^f	0.59 ± 0.04 ^f
OPVS-3	3.18 ± 0.08 ^f	2.43 ± 0.07 ^e	1.87 ± 0.09 ^f	1.00 ± 0.03 ^e	0.86 ± 0.01 ^{de}	0.72 ± 0.01 ^{de}
OPVS-4	2.92 ± 0.03 ^g	2.45 ± 0.08 ^e	1.98 ± 0.07 ^{ef}	1.14 ± 0.05 ^{cd}	1.04 ± 0.01 ^{bc}	0.82 ± 0.07 ^{cd}
OPVS-5	4.31 ± 0.09 ^c	3.67 ± 0.09 ^a	2.95 ± 0.18 ^b	1.27 ± 0.06 ^b	1.12 ± 0.08 ^{ab}	0.95 ± 0.05 ^b
OPVS-6	3.78 ± 0.03 ^{de}	3.22 ± 0.07 ^c	2.76 ± 0.04 ^{bc}	1.17 ± 0.04 ^c	1.04 ± 0.04 ^{bc}	0.91 ± 0.14 ^{bc}
OPVS-7	4.34 ± 0.01 ^c	3.19 ± 0.09 ^c	2.90 ± 0.05 ^{bc}	1.00 ± 0.01 ^e	0.79 ± 0.02 ^{ef}	0.74 ± 0.01 ^{de}
OPVS-8	3.63 ± 0.14 ^e	2.84 ± 0.11 ^d	2.48 ± 0.11 ^d	1.12 ± 0.04 ^{cd}	0.97 ± 0.01 ^{cd}	0.82 ± 0.04 ^{cd}
OPVS-9	3.84 ± 0.10 ^d	2.92 ± 0.01 ^d	2.71 ± 0.08 ^c	1.07 ± 0.02 ^{de}	0.90 ± 0.13 ^d	0.82 ± 0.08 ^{cd}
OPVS-10	4.63 ± 0.17 ^b	3.60 ± 0.08 ^{ab}	3.23 ± 0.01 ^a	1.45 ± 0.06 ^a	1.20 ± 0.01 ^a	1.08 ± 0.04 ^a
OPVS-11	5.00 ± 0.08 ^a	3.79 ± 0.15 ^a	3.35 ± 0.06 ^a	1.25 ± 0.04 ^b	0.97 ± 0.06 ^{cd}	0.89 ± 0.06 ^{bc}
OPVS-12	3.85 ± 0.04 ^d	3.41 ± 0.05 ^{bc}	2.83 ± 0.10 ^{bc}	1.28 ± 0.02 ^b	1.12 ± 0.06 ^{ab}	0.94 ± 0.03 ^{bc}

¹⁸ Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

With the application of 75% RDK, significantly higher total K-uptake (5.36 g/pot) was recorded with OPVS-12 treated pot and gave ~ 48% higher value as compared to control pot (2.78 g/pot). The KSB strains with 75% RDK showed higher efficiency than 100% RDK in terms of total K-uptake. It was justified by the higher percent increase to control in 75% RDK compared 100% RDK. In terms of total K-uptake KSB strains follow the order of OPVS-12 > OPVS-5 > OPVS-10 > OPVS-11 > OPVS-4 > OPVS-6 > OPVS-8 > OPVS-7 > OPVS-9 > OPVS-1 > OPVS-3 > OPVS-2 (Table 4.29).

Table 4.29: Effect of different KSB strains and levels of potassium on total K-uptake by maize crop¹⁹

Isolates	Total potassium uptake (g/pot)		
	100% RDK	75% RDK	50% RDK
Control	3.45 ± 0.12 ^g	2.78 ± 0.10 ^j	2.40 ± 0.08 ^e
OPVS-1	3.92 ± 0.10 ^e	3.52 ± 0.05 ^{hi}	3.13 ± 0.08 ^d
OPVS-2	3.69 ± 0.20 ^{ef}	2.85 ± 0.02 ^j	2.48 ± 0.06 ^e
OPVS-3	3.55 ± 0.18 ^f	3.30 ± 0.08 ⁱ	2.52 ± 0.10 ^e
OPVS-4	4.41 ± 0.06 ^d	4.35 ± 0.10 ^{cd}	3.10 ± 0.05 ^d
OPVS-5	5.55 ± 0.12 ^a	5.17 ± 0.01 ^{ab}	4.21 ± 0.13 ^b
OPVS-6	4.42 ± 0.18 ^d	4.21 ± 0.05 ^{de}	3.78 ± 0.06 ^c
OPVS-7	4.99 ± 0.05 ^c	3.89 ± 0.08 ^{fg}	3.66 ± 0.10 ^c
OPVS-8	4.65 ± 0.07 ^d	4.04 ± 0.05 ^{ef}	3.59 ± 0.11 ^c
OPVS-9	4.59 ± 0.05 ^d	3.70 ± 0.09 ^{gh}	3.51 ± 0.07 ^c
OPVS-10	5.56 ± 0.08 ^a	4.96 ± 0.14 ^b	4.67 ± 0.14 ^a
OPVS-11	5.18 ± 0.17 ^{bc}	4.53 ± 0.11 ^c	3.81 ± 0.01 ^c
OPVS-12	5.37 ± 0.09 ^{ab}	5.36 ± 0.08 ^a	4.21 ± 0.08 ^b

¹⁹Data are presented as mean ± standard error (n = 3), Mean followed by similar latter within a column for a particular parameter are not significantly different (P < 0.05) level of significance according to DMRT.

As regards the application of 50% RDK along with KSB strains gave significantly lower total K-uptake among the all three levels of K fertilization. The significantly higher (4.67 g/pot) total K-uptake was recorded with OPVS-10, which was ~ 49% higher as compared to uninoculated control (2.40 g/pot) followed by the OPVS-12 KSB strain (4.21 g/pot). The OPVS-10 KSB strain showed its significant superiority over the rest of the KSB strains. Data showed the values of all KSB strains, total K-uptake followed the order as OPVS-10 > OPVS-12 $\geq$ OPVS-5 > OPVS-11 > OPVS-6 > OPVS-7 > OPVS-8 > OPVS-9 > OPVS-1 > OPVS-4 > OPVS-3 > OPVS-2 (Table 4.29).

The data showed that significantly higher amount of total K-uptake was recorded with increase K fertilization with various KSB strains from 50 to 75% RDK. Additional, the ~ 15% increase was recorded with 100% RDK. These results showed that the efficiency of KSB strains was reduced with the application of higher doses of potassium. However, ~ 19% significantly higher total K-uptake was recorded with pot receiving 100% RDK as compared to 50% RDK without KSB strain (Fig. 4.37). Direct enhancement of mineral uptake due to increases in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Bashan, 1991; Bertrand *et al.*, 2000). Effect of mineral fertilizer, pig manure, and *Azospirillumrugosum* on growth promotion and nutrient contents of *Lactucasativa* L was also described by Lai *et al.* (2008).

4.16 PCA for growth, yield, nutrient uptake and nutrient availability of rhizospheric soil of maize crop

PCA is a useful statistical technique which had found application in reduction of the original variables in a smaller number of underlying variables (principal component) in order to reveal the interrelationships between the different variables and to find the optimum number of extracted principal components. Effects of various KSB and levels of K fertilization on growth, yield, nutrient content, uptake and nutrient availability in rhizospheric soil of maize crop after harvest (Fig. 4.7a and 4.37b). The PCA comprising two principal components (C1 and C2) accounted for

80.90, 77.70 and 75.30 with 10, 75 (Fig. 4.38a and 4.38b) and 50% RDK (Fig. 4.39a and 4.39b), respectively. An angle of 0 or 180° reflects a correlation of 1 or -1, respectively (Kohler and Luniak, 2005). Superimposition of KSB along with levels of K fertilization on maize crop results showed that the OPVS-5, 6, 7, 8, 9, 11 and 12 showed higher correlation with these parameters (Tables 4.37 and 4.38). The PCA of soil and plant parameters with KSB and K levels on the basis of growth, yield, nutrient content and nutrient availability comprising two principal components (C1 66.90; C2 11.20% for 100% RDK), (C1 63.70; C2 14.00% for 75% RDK) and (C1 63.70; C2 11.60% for 50% RDK) accounted for ~ 81, 77 and 75% for 100, 75 and 50% RDK of variance (Fig. 4.38).

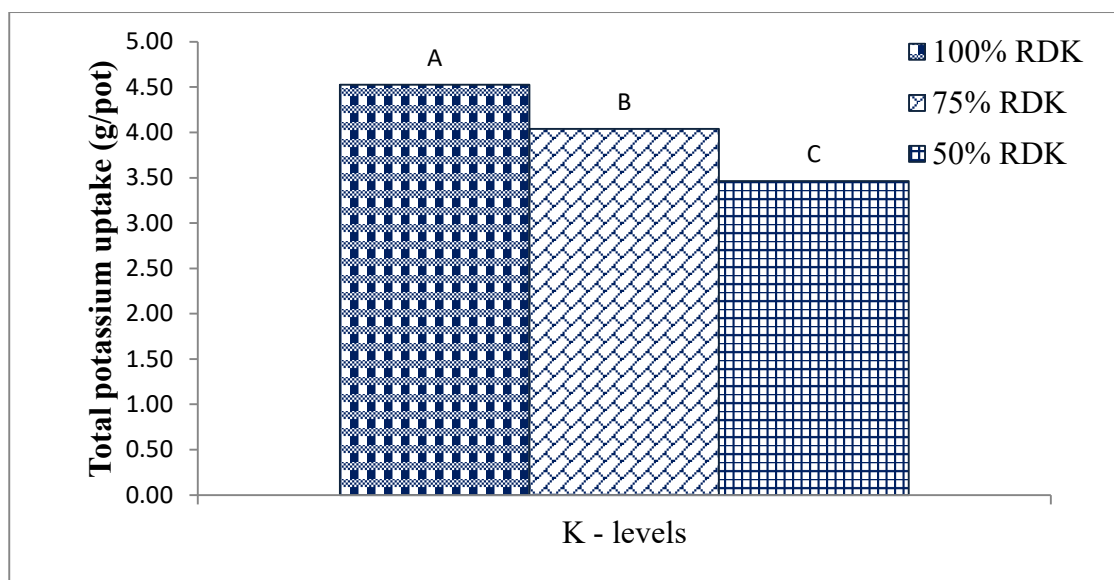


Fig. 4.36: Interaction effects of potassium solubilizing bacteria (KSB) and levels of potassium fertilization on total K-uptake by crop. Bars followed by a similar letter between treatments within a fertility levels are not significantly different at $p < 0.05$ level of significance according to Duncan's Multiple Range Test.

A position of 26 parameters in relation to their influence by plant growth and yield parameters of maize crop influenced by various KSB and varied K fertilization from ~ 81, 77 and 75 with 100, 75 and 50% RDK for various KSB, the interpretation of the PCA results can be explained by the positioning of the KSB strain group and

group of plant growth and yield component from pot experiment by superimposition of respective PCA plots for respective KSB strains.

As a result of cluster analysis, tow, three and tow clusters were obtained with 100, 75 and 50% RDK. For 100% RDK the cluster I possessed KSB of *F. anhuiense* strain OPVS05; *R. pusense* strain OPVS06; *A. tumefaciens* strain OPVS07; *F. anhuiense* strain OPVS08; *A. tumefaciens* strain OPVS09; *A. tumefaciens* strain OPVS11; *A. tumefaciens* strain OPVS12 (Tables 4.30 and 4.31), which are best discernible by their highest mean values of positive influencing plant growth, yield and nutrient uptake along with availability of nutrient in rhizospheric soil of maize crop.

For 75% RDK the cluster I possessed KSB of *R. rosettiformans* strain OPVS04; *R. pusense* strain OPVS06; *A. tumefaciens* strain OPVS07; *F. anhuiense* strain OPVS08; *A. tumefaciens* strain OPVS09; *A. tumefaciens* strain OPVS11 and for 50% RDK the I cluster possesses KSB of *F. anhuiense* strain OPVS05; *R. pusense* strain OPVS06; *A. tumefaciens* strain OPVS07; *F. anhuiense* strain OPVS08; *A. tumefaciens* strain OPVS09; *A. tumefaciens* strain OPVS11; *A. tumefaciens* strain OPVS12 was the best cluster among the all clusters (Table 4.31).

The next best cluster for 100% RDK was II, which retained *A. tumefaciens* strain OPVS01; *A. tumefaciens* strain OPVS02; *R. pusense* strain OPVS03; *R. rosettiformans* strain OPVS04; *F. anhuiense* strain OPVS08, for 75% RDK was II, which retained *R. pusense* strain OPVS03; *A. tumefaciens* strain OPVS12 and for 50% RDK was II, which retained *A. tumefaciens* strain OPVS01; *A. tumefaciens* strain OPVS02; *R. pusense* strain OPVS03; *R. rosettiformans* strain OPVS04 followed by only 75% RDK have cluster III, which retained *A. tumefaciens* strain OPVS01; *A. tumefaciens* strain OPVS02; *R. pusense* strain OPVS03 (Tables 4.37 and 4.38).

Table 4.30: Mean values of seed germination, plant growth, yield, nutrient content and uptake by crop as influenced by KSB and levels of K under net house conditions of the KSB four clusters

Crop and soil properties	Clusters						
	100% RDK		75% RDK			50% RDK	
	I	II	I	II	III	I	II
Germination (%)	91.32	93.44	92.25	93.78	93.22	91.32	93.44
Root length (cm)	10.49	7.95	9.92	9.41	7.92	10.49	7.95
Shoot length (cm)	12.11	10.46	11.94	10.75	10.00	12.11	10.46
Chlorophyll content (SPAD)	42.26	38.86	39.33	37.57	34.69	31.80	32.58
Plant height (cm)	138.10	132.22	133.37	129.01	124.31	126.63	118.75
Stem girth (cm)	5.27	3.33	4.34	3.40	2.82	3.85	2.34
Number of leaves/plant	12.76	8.75	11.56	11.17	8.33	9.90	8.17
Cob length (cm)	14.06	13.44	13.90	13.27	13.10	12.52	12.08
Grains/cob	229.24	236.67	224.50	222.33	210.78	208.33	206.00
100-grain weight (g)	25.25	22.52	22.45	23.03	21.27	21.93	20.94
Biological yield (g/plant)	394.57	328.59	351.12	362.53	294.69	343.34	277.02
Stover yield (g/plant)	250.13	197.70	220.33	231.97	172.89	217.39	158.31
Grain yield (g/plant)	144.44	130.89	130.80	130.56	121.80	125.95	118.71
Harvest index (%)	36.65	39.98	37.32	36.68	41.47	36.75	43.08
Available nitrogen (mg/kg)	113.41	110.06	107.17	109.04	108.17	99.01	100.19
Available phosphorus (mg/kg)	11.39	10.66	10.39	10.25	9.20	9.73	8.46
Available potassium (mg/kg)	110.34	108.46	91.64	87.66	89.17	69.06	73.11
N uptake by grain (g/pot)	2.20	1.71	1.72	1.66	1.39	1.53	1.22
P uptake by grain (g/pot)	0.61	0.52	0.49	0.48	0.40	0.43	0.37
K uptake by grain (g/pot)	0.64	0.54	0.53	0.50	0.44	0.48	0.40
N uptake by stover (g/pot)	1.91	1.35	1.35	1.27	1.00	1.32	0.80
P uptake by stover (g/pot)	0.56	0.46	0.46	0.51	0.37	0.44	0.33
K uptake by stover (g/pot)	4.33	3.36	3.59	3.83	2.78	3.34	2.41
Total N uptake by crop (g/pot)	4.11	3.06	3.07	2.92	2.39	2.85	2.02
Total P uptake by crop (g/pot)	1.17	0.98	0.95	0.99	0.77	0.87	0.71
Total K uptake by crop (g/pot)	4.96	3.89	4.12	4.33	3.22	3.82	2.81

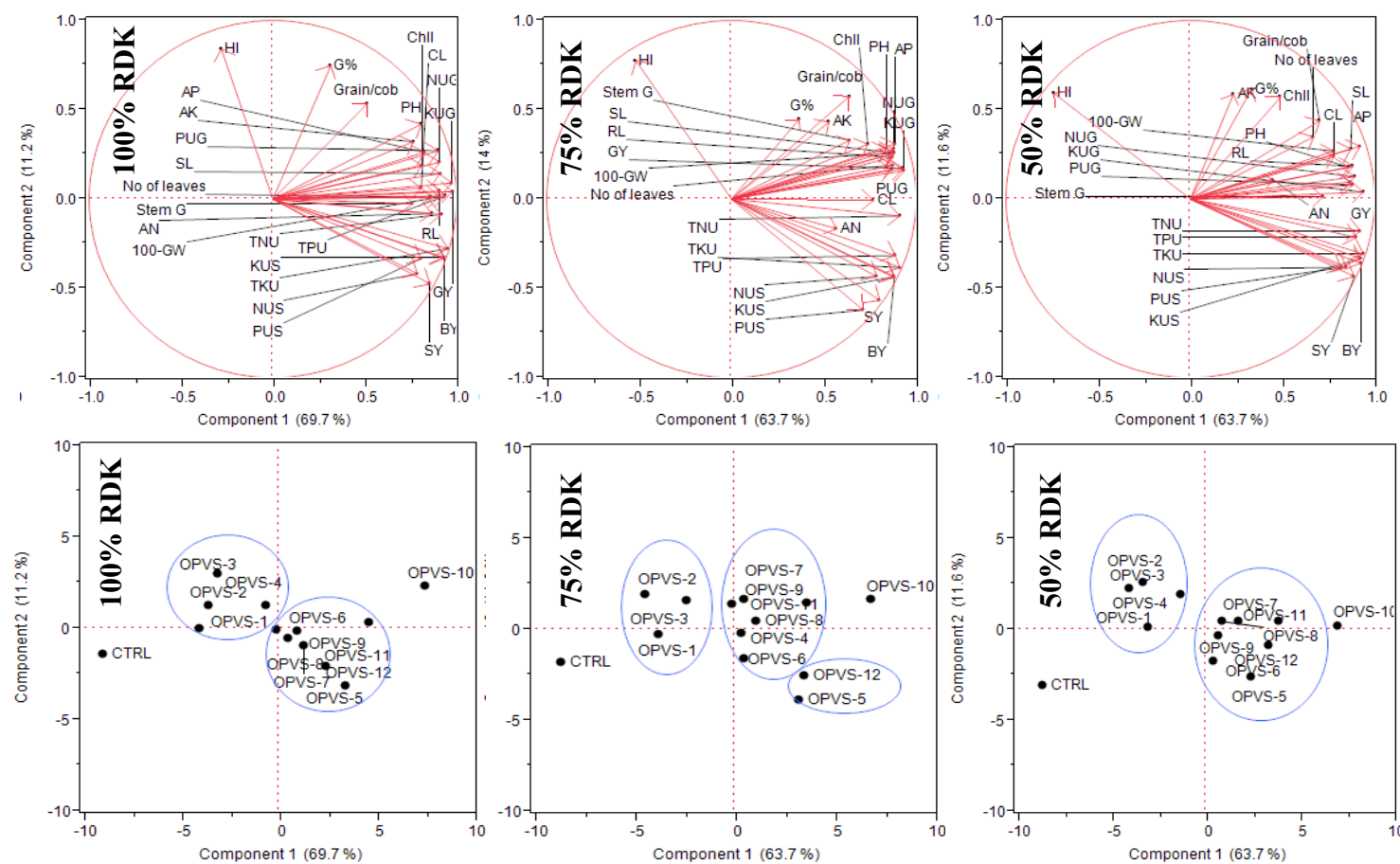


Fig. 4.37: Loading plot and grouping of KSB on the basis of PCA scores of KSB with level of K-fertilization (For KSB strain details see the materials and methods section)

Table 4.31: Composition of clusters of 11 KSB activities with levels of potassium based on the value of PCA

	Cluster no.	No. of KSB	Name of KSB species
100% RDK	I	7	<i>F. anhuiense</i> strain OPVS05; <i>R. pusense</i> strain OPVS06; <i>A. tumefaciens</i> strain OPVS07; <i>F. anhuiense</i> strain OPVS08; <i>A. tumefaciens</i> strain OPVS09; <i>A. tumefaciens</i> strain OPVS11 <i>A. tumefaciens</i> strain OPVS12
	II	4	<i>A. tumefaciens</i> strain OPVS01; <i>A. tumefaciens</i> strain OPVS02; <i>R. pusense</i> strain OPVS03; <i>R. rosettiformans</i> strain OPVS04
75% RDK	I	6	<i>R. rosettiformans</i> strain OPVS04; <i>R. pusense</i> strain OPVS06; <i>A. tumefaciens</i> strain OPVS07; <i>F. anhuiense</i> strain OPVS08; <i>A. tumefaciens</i> strain OPVS09; <i>A. tumefaciens</i> strain OPVS11
	II	2	<i>R. pusense</i> strain OPVS03; <i>A. tumefaciens</i> strain OPVS12
	III	3	<i>A. tumefaciens</i> strain OPVS01; <i>A. tumefaciens</i> strain OPVS02; <i>R. pusense</i> strain OPVS03 <i>F. anhuiense</i> strain OPVS05; <i>R. pusense</i> strain OPVS06; <i>A. tumefaciens</i> strain OPVS07; <i>F. anhuiense</i> strain OPVS08; <i>A. tumefaciens</i> strain OPVS09; <i>A. tumefaciens</i> strain OPVS11 <i>A. tumefaciens</i> strain OPVS12
50% RDK	I	7	<i>A. tumefaciens</i> strain OPVS01; <i>A. tumefaciens</i> strain OPVS02; <i>R. pusense</i> strain OPVS03; <i>R. rosettiformans</i> strain OPVS04
	II	4	

SUMMARY AND CONCLUSIONS

5.1 Summary

Potassium is released from the slowly or non-exchangeable K pool from the lattice wedge sites of weathered micaceous clay minerals which are selective for K ions. The remaining pool or major pool of K which holds the bulk 90-98% of the total soil K, is held in structure of the primary K bearing minerals soils, K occurs in the form of silicate minerals viz., muscovite, orthoclase, biotite, feldspar, illite, mica (ruby mica, mica powder, mica scrap, mica stone, mica flakes), vermiculite, smectite etc. Total pool of soil K which is extremely complex can be solubilized by the KSB in the available form to the crops. Keeping these in view this work entitled **“Isolation, Characterization and Screening of Potassium-Solubilizing Bacteria from Soils of Varanasi”** was carried out with the following objectives.

1. Isolation of potassium solubilizing bacteria from rhizospheric soils of Varanasi district.
2. Screening of K-solubilizing isolates on basis of K-solubilizing capacity.
3. Physiological and biochemical characterization of K-solubilizing bacteria.
4. Periodical study on release of K from K-minerals by some virulent K-solubilizing isolates, and
5. To study the production of growth promoting substances by K-solubilizing isolates and their effect on seed germination, growth and yield of maize.

A total of 95 rhizospheric soil samples from eight blocks of Varanasi district were collected from dominating crops such as maize (*Z. mays*), banana (*M. paradisiaca*), tobacco (*N. tabacum*), sugarcane (*S. officinarum*), pigeon pea (*C. cajan*), and potato (*S. tuberosum*). All cropped fields were located in Indo-Gangetic

Plains of the holy city Varanasi (El 80.71 m [264.80 ft]; Lt25.3333⁰ N; Lg 83.0000⁰ E) which is also known by the names as Banaras/Kashi in the state of Uttar Pradesh, North Central Part of India.

5.1.1: Isolation and screening of KSB

1. A total of 127 KSB isolates were isolated from rhizospheric soil of six crops of Varanasi district. Isolates were screened on the basis of their K-solubilization capacity ranged from 20.72 - 23.13 µg/mL.
2. K-solubilization capacity of isolates was studied *in-vitro* using waste muscovite supplemented broth. The K-solubilization of selected isolates ranged from 7.16 - 23.13 µg/mL. Twelve isolates on merit of their greater K-solubilization capacity were taken for onward studies.
3. Based on 16S phylogeny, out of twelve isolates affirmed species of KSB, seven isolates (58.33%) were found to be closely phylogenetically related to *Agrobacterium* showing about 99-100% similarity in their 16S rDNA sequences, making *Agrobacterium* the most dominant genus namely *Agrobacterium tumefaciens* strain (58.33%). There were 2, 2 and 1 isolates which belong to *Rhizobium pusense* strain (16.66%), *Flavobacterium anhuiense* strain (16.66%), and *Rhizobium rosettiformans* strain (8.33%), respectively.

5.1.2: Characterization of KSB

1. Out of twelve, nine KSB isolates showed gram positive and the rest three isolates were gram negative in reaction. The zone of solubilization was significantly higher with OPVS 11 (2.34 cm) as compared to rest of the strains.
2. Six KSB isolates showed positive test to starch hydrolysis. All strains were acid producers and five strains produced H₂S gas. The KSB strains OPVS-1, 3, 6, 8, 1, and 12 resulted positive to methyl red (MR) test and five strains OPVS-1, 3, 6, 9 and 12 showed positive reaction to VP test.

Summary and Conclusions

3. Five KSB strains (OPVS-1, 3, 6, 9 and 12) produced GA_3 and NH_3 , eight HCN (OPVS-2, 3, 5, 6, 7, 9, 11 and 12), and all KSB strain showed positive oxidase test.
4. Significantly higher IAA production amounting 25, 50, 75 and 100 $\mu\text{g/mL}$ of tryptophan was recorded with OPVS-4, 10, 8 and OPVS-10 strain, respectively.
5. All 12 KSB isolates significantly influenced K release and pH dynamic in broth culture of waste muscovite and biotite broth at 7, 14 and 21 DAI. *A. tumefaciens* OPVS-11 followed by *R. pusense* OPVS-6 significantly reduced pH of medium indicated acidolysis as one of the chief mechanisms of the KSB isolates.
6. K-release dynamics from waste muscovite and biotite was significantly influenced by *A. tumefaciens* OPVS-1 and *A. tumefaciens* OPVS-2 in comparison to rest of the KSB strains.
7. Maximum and significantly higher KSB cell growth was recorded at 30°C which was $\sim 66, 26, 22, 49$ and 68% higher at the temperature $20, 25, 35, 40$ and 45°C , respectively as compared to control. The regression equation for the quadratic response to KSB cell growth with temperature was $y = -0.002\text{Temp}^2 + 0.147\text{Temp} - 1.780$, $R^2 = 0.881$.
8. At the pH 7.6 KSB cell growth were $\sim 49, 25, 15, 33$ and 42% with 6.4, 7.0, 7.6, 8.2, 8.8 and 9.4 pH, respectively, with $y = -0.078\text{pH}^2 + 1.241\text{pH} - 4.485$, $R^2 = 0.84$ regression equation.
9. Growth of all the KSB strains decreased with increasing concentration of salt and the regression equation for the quadratic response was $y = 0.000\text{NaCl}^2 - 0.037\text{NaCl} + 0.571$, $R^2 = 0.972$. The highest KSB cell growth was recorded with concentration of 1% NaCl. It was $\sim 14, 25, 30, 40$ and 52% with 2, 4, 6, 8 and 10% NaCl, respectively.
10. The PCA of K-solubilization activities from waste mica (muscovite and biotite) comprising two principal components (C1-33.9; C2-18.7 and C1-36.3; C2-18.0% for KSB) accounted for ~ 53 and 55% , for muscovite and biotite, respectively.

5.2: Nethouse experiment with maize crop

In the second part of study effect of twelve KSB strains along with levels of K fertilization was studied on growth and yield attributes of maize under nethouse condition. The experimental soil was fine loamy illitic, hyperthermic Typic Haplustepts which had bulk density 1.39 Mg/m³, particle density 2.56 Mg/m³, W.H.C. 40.27%, pH 7.4, EC 0.44 dSm⁻¹, wet oxidizable organic C 4.9 gkg⁻¹, available N 97.80 mgkg⁻¹, available P₂O₅ 7.54 mgkg⁻¹ and available K₂O 83.50 mgkg⁻¹.

The nethouse experiment consists of three levels of K fertilization (100, 75 and 50% RDK) and twelve KSB strains (*A. tumefaciens* strain OPVS01, *A. tumefaciens* strain OPVS02, *R. pusense* strain OPVS03, *R. rosettiformans* strain OPVS04, *F. anhuiense* strain OPVS05, *R. pusense* strain OPVS06, *A. tumefaciens* strain OPVS07, *F. anhuiense* strain OPVS08, *A. tumefaciens* strain OPVS09, *A. tumefaciens* strain OPVS10, *A. tumefaciens* strain OPVS11 and *A. tumefaciens* strain OPVS12) along with one set of control pots. The recommended dose of fertilizers was used as 120, 60 and 60 kg/ha (N, P₂O₅ and K₂O, respectively). 1/3rd of N and full P and K were applied as basal. Remaining dose of total N was applied in two equal splits at 30 & 60 days after sowing.

A total 39 treatments combination replicated in thrice under FCRD with maize cultivar MHM-2. Seeds were inoculated with KSB strains 10⁸ cfu/mL before sowing and sown in 10 kg capacity of earthen pots filled with processed soil. The salient features of the experimental findings emanating from the experiment are summarized as below:

1. Germination of seeds varied from 85.89 to 95.49% with various KSB strains. Significantly higher (95.49%) seed germination was recorded with KSB strains OPVS-3 and it was ~ 11% higher as compared to uninoculated control pot (85.89%).

Summary and Conclusions

2. Significantly higher shoot and root length 15.56 and 13.58 cm, respectively, were recorded with OPVS-10 which caused ~ 4 fold higher germination of seeds as compared to control.
3. Significantly higher plant height (156.01 cm) of maize at 60 DAS was recorded due to 100% RDK with OPVS-10 isolates followed by 75% and 50% RDK.
4. Stem girth varied from 2.63 to 6.57 cm with various KSB strains and followed the order as OPVS-10 > OPVS-7 > OPVS-9 > OPVS-8 > OPVS-12 > OPVS-6 > OPVS-11 > OPVS-2 > OPVS-1 > OPVS-5 > OPVS-4 > OPVS-3.
5. The content of chlorophyll was significantly higher (46.30 SPAD) with application of 100% RDK and OPVS-9 which showed its significantly superiority over rest of the KSB strains except OPVS-4, 10 and 11. Results showed that efficiency of KSB strains was reduced with application of higher doses of potassium. However, ~ 32% higher chlorophyll content was recorded with pot receiving 100% RDK as compared to 50% RDK.
6. Application of 75% RDK caused ~ 6% greater leaves per plant over 50% RDK. However, in case of 100% RDK ~ 9% higher levels were recorded as compared to 75% RDK.
7. Pots with OPVS-10 produced longest (14.94 cm) cob which was ~ 21% higher as compared to control. Application of 100% RDK produced ~ 19% longer cob as compared to 50% RDK.
8. Grain per cob significantly influenced by application of 75% RDK which caused ~ 4% and 19% higher grains per cob than 50% RDK and 100% RDK respectively. With doses of K-application OPVS-10 gave higher grain per cob than OPVS-11.
9. KSB stains caused significantly higher 100-grain weight as compared to control. 75% RDK gave ~ 11% higher grain weight when compared with 50% RDK. The application of 100% RDK gave ~ 13 and 26% higher grain weight as compared to

75 and 50% RDK, respectively. OPVS-10 with 100% RDK yielded highest and significantly more grains than control.

10. The OPVS-5 gave significantly higher stover yield, which was ~ 49% higher than uninoculated control. More than 7% greater stover yield was recorded with pot receiving 100% RDK as compared to 50% RDK.

5.2.1: Maize rhizospheric nutrient (NPK) availability

1. Significantly higher available N (~ 117 mg/kg) was recorded with OPVS-5 in combination with 100% RDK as compared to 75 and 50% RDK.
2. KSB strains OPVS-8 in combination with 75% RDK showed significantly higher content of available N (~ 116mg/kg) as compared to 50% RDK.
3. Significantly higher (~ 11 mg/kg) available P was recorded with 100% RDK as compared to 75% RDK (~ 10 mg/kg) and 50% RDK (~ 9 mg/kg) with various KSB strains.
4. Maximum content of available K was recorded with 100% RDK.
5. OPVS-10 in combination with 100% RDK gave significantly higher available K as compared to 75 and 50% RDK. This combination showed significant superiority by ~ 17, 13, 14% over 100, 75 and 50% RDK, respectively.

5.2.2: Nutrients uptake by grain and stover of maize

1. 100% RDK caused ~ 20 and 31% higher N uptake by grain and 21 and 32 % higher N uptake by stover in comparison to 75 and 50% RDK, respectively.
2. P uptake by grain was significantly higher with 100% RDK (0.58 g/pot) as compared to 75% RDK (0.47 g/pot) and 50% RDK (0.41 g/pot).
3. Application of 100 and 75% RDK in combination with OPVS-5 caused ~ 35 and 41% higher P uptake by stover of maize as compared to control.

4. Inoculation of OPVS-10 with 50% RDK resulted ~ 43% higher P uptake by stover of maize crop.
5. 100% RDK gave ~ 10 and 23% significantly higher P uptake by stover with 75 and 50% RDK, respectively.
6. Application of 100% RDK gave significantly higher K uptake as compared to 75% RDK and 50% RDK.
7. Application of 100% RDK in combination with OPVS-10 caused significantly ~ 46% higher K uptake by grain as compared to control.
8. The OPVS-5 in combination with doses of RDK showed its significant superiority over the rest of KSB strains.

5.2.3: Total NPK uptake by maize crop

1. Increasing the K level from 50 to 75%, with OPVS- 11 gave ~ 13%, higher total N-uptake and further addition of 25% K (100%), caused ~ 32 percent more total N-uptake by maize crop. OPVS- 11 with 100% RDK gave ~ 49% more N-uptake by crop than obtained with 50% RDK.
2. Levels of K fertilization without KSB strains, though caused a marked effect on total P-uptake, but values were relatively lower as compared to KSB strains. Successive increase in recommended doses of potassium caused significantly higher total P-uptake by maize crop.
3. Significantly higher total K-uptake (5.56 g/pot) was recorded with 100% RDK + OPVS-10. This showed its significant superiority over rest of the KSB strains except OPVS-5 and 12. KSB, OPVS-10 gave ~ 38%, significantly higher total K-uptake as compared to control (3.45 g/pot) pot.
4. Application of 75% RDK with OPVS-12, gave significantly 48% higher total K-uptake (5.36 g/pot) as compared to control (2.78 g/pot).

5. Increasing levels of RDK along with KSB isolates gave significantly higher total uptake of K. The significantly higher (4.67 g/pot) total K-uptake was recorded with OPVS-10 which was ~ 49% higher as compared to uninoculated control (2.40 g/pot) followed by OPVS-12 (4.21 g/pot).
6. Efficiency of KSB strains reduced with higher doses of potassium. However, ~ 19%, higher total K-uptake was recorded with pot received 100% RDK as compared to 50% RDK.

5.3: Multivariate analysis

1. Effect of various KSB isolates and levels of K fertilization on growth, yield, content as well as uptake of nutrients and fertility status of rhizospheric soil of maize after harvest were analyzed. The PCA comprising two principal components (C1 and C2) accounted for 80.90, 77.70 and 75.30 with 100, 75 and 50% RDK, respectively.
2. Soil and plant parameters with KSB and K levels on the basis of growth, yield, nutrient content and nutrient availability comprising two principal components (C1 66.9; C2 11.2% for 100% RDK), (C1 63.7; C2 14.0% for 75% RDK) and (C1 63.7; C2 11.6% for 50% RDK) accounted for ~ 81, 77 and 75% for 100, 75 and 50% RDK of variance, respectively. These results indicated that OPVS-7 is an efficient acid- and OPVS-9 is an efficient alkali-tolerant KSB isolates which can be applied in acidic and alkaline nature of soils, respectively.

5.4: Conclusions

Twelve screened isolates used in nethouse pot experiment with maize crop belong to 4 different species of KSB based on 16S phylogeny. They were *Agrobacterium tumefaciens* strain (58.33%), *Rhizobium pusense* strain (16.66%), *Flavobacterium anhuiense* strain (16.66%), and *Rhizobium rosettiformans* strain (8.33%). The KSB strain OPVS-11 followed by OPVS-6 significantly reduced pH of MAB and K-release dynamics from waste muscovite and biotite. Temperature as 30°C, pH 7.6 and 1% NaCl concentration were recorded as suitable for optimum

Summary and Conclusions

growth of KSB isolates. KSB strains OPVS-10 and 11 showed their significant superiority over rest of the KSB strain on crop growth, yield attributes content and uptake of nutrients with 100% RDK followed by 75 and 50% RDK. The KSB isolates OPVS-11 followed by OPVS-6 significantly enhanced K-release dynamics from both waste muscovite and biotite. Temperature as 30⁰C, pH 7.6 and 1% NaCl concentration were recorded as suitable for optimum growth of KSB isolates. It was observed that efficiency of KSB isolates on growth, yields and K-uptake of maize crop increased with decreasing in doses of potassium. Application of KSB isolates OPVS-11, 10 and 4 emerged out as the most efficient isolates. These isolates performed well with 75% RDK.

Application of KSB and recommended levels of K significantly influenced the yield of maize crop and nutrient availability as compared to uninoculated control. The plant growth promoting activities of KSB strains were also enhanced, especially with 75 and 50% RDK. Results indicated that OPVS-7 and OPVS-9 emerged out as efficient acid and alkali-tolerant KSB isolates, respectively. Use of these efficient KSB isolates under favourable conditions certainly will enhance supply of K to crop. However, authentications through multilocation field trails are necessary prior the recommendation of these KSB isolates as Potassic inoculants.

BIBLIOGRAPHY

- Abdel-Salam, M. and Shams, A. (2012) Feldspar-K fertilization of potato (*Solanum tuberosum* L.) augmented by biofertilizer. *Journal of Agriculture and Environmental Sciences* **12**, 694-699.
- Abou-el-Seoud, I.I. and Abdel-Mageed, A. (2012) Impact of rock materials and biofertilization on P and K availability for maize (*Zea mays*) under calcareous soil conditions. *Saudi Journal of Biological Sciences* **19**, 55-63.
- Adesemoye, A.O., Torbert, H.A. and Kloepper, J.W. (2008) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology* **54**, 876-886.
- Ahmad, F., Ahmad, I. and Khan, M.S. (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* **163**, 173-181.
- Ahmad, M., Zahir, Z.A., Asghar, H.N. and Arshad, M. (2012) The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. *Annals of Microbiology* **62**, 1321-1330.
- Aleksandrov, V., Blagodyr, R. and Ilev, I. (1967) Liberation of phosphoric acid from apatite by silicate bacteria. *Microbiology* **29**, 111-114.
- Aleksandrov, V.G. (1958) Organo-mineral fertilizers and silicate bacteria. *Dokl Akad. S.KhNauk* **7**, 43-48.
- Ali, B. and Hasnain, S. (2007) Efficacy of bacterial auxin on in-vitro growth of *Brassica oleracea* L. *World Journal of Microbiology and Biotechnology* **23**, 779-784.
- Altomare, C., Norvell, W.A., Björkman T. and Harman, G.E. (1999) Solubilization of phosphates and micronutrients by the plantgrowth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied Environment and Microbiology* **65**: 2926-2933.

- Alves, L., Oliveira, V.L., and Filho, G.N.S. (2010) Utilization of rocks and ectomycorrhizal fungi to promote growth of eucalypt. *Brazilian Journal of Microbiology* **41**, 76-84.
- Aneja, K.R. (2003) Experiments in Microbiology, Plant pathology and Biotechnology, 4th Edition. New Age International Publishers, Daryaganj, New Delhi.
- Anonymous (2014) Agriculture at a glance – Maize. Department of Agriculture, Government of Punjab, available at www.agripb.gov.in/ext. accessed on 4 July 2014.
- Archana, D.S., Nandish, M.S., Savalagi, V. and Alagawadi, A. (2013) Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil. *Bioinfolet* **10**, 248-257.
- Archana, D.S., Nandish, M.S., Savalagi, V.P. and Alagawadi, A.R. (2012) Screening of potassium solubilizing bacteria (KSB) for plant growth promotional activity. *Bioinfolet* **9**, 627-630.
- Archana, D.S., Savalgi, V.P. and Alagawadi, A.R. (2008) Effect of potassium solubilizing bacteria on growth and yield of maize. *Soil Biological Ecology* **28**:9-18.
- Argelis, D.T., Gonzala, D.A., Vizcaino, C. and Gartia, M.T. (1993) Biochemical mechanism of stone alteration carried out by filamentous fungi living in monuments. *Biogeochemistry* **19**, 129-147.
- Armengaud, P., Breitling, R. and Amtmann, A. (2010) Coronatine-intensive 1 (COII) mediates transcriptional responses of *Arabidopsis thaliana* to external potassium supply. *Molecular Plant* **3**, 390-405.
- Arruda, L., Beneduzi, A., Martins, A., Lisboa, B., Lopes, C., Bertolo, F. and Vargas, L.K. (2013) Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. *Applied Soil Ecology* **4**, 15-22.
- Asghar, H.N., Zahir, Z.A., Arshad, M. and Khaliq, A. (2002) Relationship between *in-vitro* production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils* **35**, 231-237.

- Ashley, D.L., Blount, B., Singer, P.C., Depaz, E., Wilkes, C. and Gordon, S. (2005) Changes in blood trihalomethane concentrations resulting from differences in water quality and water-use activities. *Archives of Environmental and Occupational Health* **60**(1), 7-15.
- Askegaard, M., Eriksen, J. and Johnston, A.E. (2004) Sustainable management of potassium. In: Schjorring P, Elmholt S, Christensen BT (eds) Managing soil quality: challenges in modern agriculture, Wallingford, CABI Publishing, pp. 85-102.
- Awasthi, R., Tewari, R. and Nayyar, H. (2011) Synergy between plants and P-solubilizing microbes in soils: effects on growth and physiology of crops. *International Research Journal of Microbiology* **2**, 484-503.
- Babalola, O.O. (2010) Beneficial bacteria of agricultural importance. *Biotechnology Letters* **32**, 1559-1570.
- Babalola, O.O., Berner, D.K. and Amusa, N.A. (2007) Evaluation of some bacterial isolates as germination stimulants of *Striga hermonthica*. *African Journal of Agricultural Research* **2**, 27-30.
- Babatola, I.A. (2006) Effects of NPK 15:15:15 fertilizer on the performance and storage life of okra (*Abelmoschus esculentus*). *Proceedings of the Horticultural Society of Nigeria Conference*, pp. 125-128.
- Badar, M.A. (2006) Efficiency of K feldspar combined with organic material and silicate dissolving bacteria on tomato yield. *Journal of Applied Science and Research* **2**, 1191-1198.
- Badar, M.A., Shafei, A.M. and Sharaf, El-Deen. S.H. (2006) The dissolution of K and phosphorus bearing minerals by silicate dissolving bacteria and their effect on sorghum growth. *Research Journal of Agricultural Biological Sciences* **2**, 5-11.
- Bagyalakshmi, B., Ponmurugan, P. and Marimuthu, S. (2012) Influence of potassium solubilizing bacteria on crop productivity and quality of tea (*Camellia sinensis*). *African Journal of Agricultural Research* **7**, 4250-4259.
- Bahadur, I., Meena, V.S., and Kumar, S. (2014) Importance and application of potassic biofertilizer in Indian agriculture. *International Research Journal of Biological Sciences* **3**(12), 80-85.

- Bakker, A.W. and Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp mediated plant growth stimulation, *Soil Biology and Biochemistry* **19**, 451-457.
- Baldi, E., Toselli, M., Eissenstat, D.M. and Marangon, B. (2010) Organic fertilization leads to increased peach root production and lifespan. *Tree Physiology* **30**, 1373-1382.
- Basak, B. and Biswas, D. (2012) Modification of waste mica for alternative source of potassium: evaluation of potassium release in soil from waste mica treated with potassium solubilizing bacteria (KSB). *LAMBERT Academic Publishing, Germany* ISBN-13:978-3659298424
- Basak, B.B. and Biswas, D.R. (2009) Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. *Plant and Soil* **317**, 235-255.
- Basak, B.B. and Biswas, D.R. (2010) Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. *Biological and Fertility of Soils* **46**, 641-648.
- Bashan, Y. (1991) Changes in membrane potential of intact soybean root elongation zone cells induced by *Azospirillum brasilense*. *Canadian Journal of Microbiology* **37**, 958-963.
- Bennett, P.C., Choi, W.J. and Rogera, J.R. (1998) Microbial destruction of feldspars. *Mineral Management* **8**(62A), 149-150.
- Bent, E.S., Tuzun, C.P. Chanway and S. Enebak, (2001) Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Canadian Journal Microbiology* **47**, 793-800.
- Bertrand, H., Plassard, C., Pinochet, X., Touraine, B., Normand, P. and Cleyet-Marel, J.C. (2000) Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium (*Achromobacter* spp.). *Canadian Journal of Microbiology* **46**, 229-236.

- Bin, L., Bin, W., Mu, P., Liu, C. and Teng, H.H. (2010) Microbial release of potassium from K-bearing minerals by thermophilic fungus *Aspergillus fumigatus*. *Geochimical et Cosmochimistry Acta* **72**, 87-98.
- Biswas, D.R. (2011) Nutrient recycling potential of rock phosphate and waste mica enriched compost on crop productivity and changes in soil fertility under potato–soybean cropping sequence in an Inceptisol of Indo-Gangetic Plains of India. *Nutrient Cycling in Agroecosystems* **89**, 15-30.
- Biswas, D.R. and Basak, B.B. (2014) Mobilization of potassium from waste mica by potassium-solubilizing bacteria (*Bacillus mucilaginosus*) as influenced by temperature and incubation period under in vitro laboratory conditions. *Agrochimica*, **58**(4). DOI 10.12871/0021857201442.
- Bossemeyer, D. Schlosser, A. and Baker, E.P. (1989) Specific cesium transport via the *Escherichia coli* (Kup (TrkD) K⁺ uptake system. *Journal of Bacteriology* **171**, 2219-2221.
- Brick, J.M., Bostock, R.M. and Silverstone, S.E. (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Applied and Environment Microbiology* **57**, 535-538.
- Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E. and Schulze-Lefert, P. (2013) Structure and functions of the bacterial microbiota of plants. *Annul Review of Plant Biology* **64**, 807-838.
- Caballero-Mellado, J., Martínez-Aguilar, L., Paredes-Valdez, G. and Estrada-de los Santos, P. (2004) *Burkholderia unamae* sp nov., an N₂-fixing rhizospheric and endophytic species. *International Journal of Systematic and Evolutionary Microbiology* **54**, 1165-1172.
- Cappuccino, J.C. and Sherman, N. (1992) In *Microbiology: A Laboratory Manual*, New York, 125-179.
- Chaiharn, M. and Lumyong S. (2011) Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. *Current Microbiology* **62** (1), 173-181.
- Chopra, S.L., Kanwar, J.S. (1982) In *analytical agricultural chemistry*, Kalyani Publishers, New Delhi, p. 337.

- Christensen, W.B. (1946) Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from salmonella and shigella types. *Journal Bacteriological Octa* **52**(4), 461-466.
- Clark, R.B. and Zeto, S.K. (1996) Growth and root colonization of mycorrhizal maize grown on acid and alkaline soil. *Soil Biology and Biochemistry* **28**,1505-1511.
- Clark, R.B. and Zeto, S.K. (2000) Mineral acquisition by arbuscular mycorrhizal plants. *Journal Plant Nutrient* **23**, 867-902.
- Clark, R.B., Zobel, R.W. and Zeto, S.K. (1999) Effects of mycorrhizal fungus isolate on mineral acquisition by *Panicum virgatum* in acidic soil. *Mycorrhiza* **9**,167-176.
- Claus, D. and Berkeley, C.W. (1986) The genus *Bacillus*. In: Bergey's Manual of Systematic Bacteriology. Vol **2**. Sneath PHA (Ed). Williams, Wilkins, Baltimore **34**, 1105-1139.
- Coroneos, C., Hinsinger, P. and Gilkes, R.J. (1996) Granite powder as a source of potassium for plants: a glasshouse bioassay comparing two pasture species. *Fertilizer Research* **45**, 143-152.
- Das, B.K., Sen, S.P. (1981) Effect of nitrogen, phosphorus and potassium deficiency on the uptake and mobilization of ions in Bengal gram (*Cicer arietinum*). *Journal of Bioscience* **3**, 249-258.
- Diep, C.N. and Hieu, T.N. (2013) Phosphate and potassium solubilizing bacteria from weathered materials of denatured rock mountain, Ha Tien, KiênGiang province Vietnam. *American Journal of Life Science Research* **1**, 88-92.
- Domínguez-Ferreras, A., Munoz, S., Olivares, J., Soto, M.J., Sanjuan, J. (2009) Role of potassium uptake systems in *Sinorhizobium meliloti* adaptation and symbiotic performance. *Journal of Bacteriology* **21**, 33–43.
- Duncan, D.B. (1955) Multiple range and multiple F-tests. *Biometrics* **11**, 1-42.
- Egamberdiyeva, D. and Hoflich, C. (2003) Influence of growth promoting bacteria on the growth of wheat in different soils and temperatures. *Soil Biology and Biochemistry* **35**, 973-978.
- Egamberdiyeva, D. (2006) Enhancement of wheat performance with plant growth promoting bacteria in different soils. In: Mukerji KG, Manoharachary C (eds), *Current Concepts in Botany*, International Publishing House Ltd 417–425.

- El-Mesbahi, M.N., Azcón, R., Ruiz-Lozano, J.M. and Aroca, R. (2012) Plant potassium content modifies the effects of arbuscular mycorrhizal symbiosis on root hydraulic properties in maize plants. *Mycorrhiza* **22**, 555-564.
- Epstein, W. (2003) The roles and regulation of potassium in bacteria. *Prog Nucleic Acid. Research on Molecular Biology* **75**, 293–320.
- Epstein, W., Kim, B.S. (1971) Potassium transport loci in *Escherichia coli* K-12. *Journal of Bacteriology* **108**, 639-44.
- Foth, H.D. and Ellis, B.G. (1997) Soil Fertility. CRC Press, Boca Raton, Florida. pp. 290.
- Friedrich, S., Platonova, N.P., Karavaiko, G.I., Stichel, E. and Glombitza, F. (2004) Chemical and microbiological solubilization of silicates. *Acta Biotechnology* **11**, 187-196.
- Gahoonia, T.S., Care, D. and Nielsen, N.E. (1997) Root hairs and phosphorus acquisition of wheat and barley cultivars. *Plant and Soil* **191**, 181-188.
- Gerhardt, P., Murray, R.G.E., Costilow, Nester, R.N. E.W., Wood, W.A., Krieg, N.R. and Phillips, G.B. (1981) Manual of methods for general bacteriology. *American Society for Microbiology*, Washington D.C., WA, USA.
- Gholami, A., Shahsavani, S. and Nezarat, S. (2009) The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of Maize. *World Academic of Science Engineering and Technology* **49**, 19-24.
- Gillus, R.P. (1956) An evaluation of two composite media for preliminary identification of *Shigella* and *Salmonella*. *Journal of Clinical Pathology* **9**, 368-371.
- Girgis, M.G.Z., Khalil, H.M.A. and Sharaf, M.S. (2008) *In-vitro* evaluation of rock phosphate and potassium solubilizing potential of some *Bacillus* strains. *Australian Journal Basic and Applied Science* **2** (1), 68–81.
- Goldstein, A.H. (1994) Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. Phosphate in microorganisms: cellular and molecular biology. *ASM Press Washington DC*. p. 197-203.
- Govindarajan, M., Kwon, S.W. and Weon, H.Y. (2007) Isolation, molecular characterization and growth promoting activities of endophytic sugarcane

- diazotroph *Klebsiella* spp. GR9. *World Journal of Microbiology and Biotechnology* **23**, 997-1006.
- Grichko, V.P. and Glick, B.R. (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiological and Biochemistry* **39**, 11-17.
- Groudev, S.N. (1987) Use of heterotrophic microorganisms in mineral biotechnology. *Acta Biotechnology* **7**, 299-306.
- Groudeva, V.I., Groudev, S.N. (1987) Aluminosilicate biodegradation in the soil. In: Proceedings of the 9th International Symposium on Soil Biology and Conservation of the Biosphere (Szegi J., ed). Akademiai Kiado Budapest, pp. 621-628.
- Gundala, P.B., Chinthala, P., Sreenivasulu, B. (2013) A new facultative alkaliphilic, potassium solubilizing, *Bacillus* spp. SVUNM9 isolated from mica cores of Nellore district, Andhra Pradesh, India. *Journal of Microbiology and Biotechnology* **2**(1), 1-7.
- Han, H.S. and Lee, D.K. (2005) Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. *Research Journal of Agricultural and Biological Science* **1**, 176-180.
- Han, H.S., Supanjani, E. and Lee, K.D. (2006) Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil Environment* **52**(3), 130-136.
- Hanway, J.J. and Heidel, H. (1952) Soil analysis methods as used in Iowa state college, soil testing laboratory. *Iowa Agriculture* **54**, 1-31.
- Hariprasad, P. and Niranjana, S.R. (2009) Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant and Soil* **316**, 13-24.
- Hasan, R. (2002) Potassium status of soils in India. *Better Crops* **16**(2), 3-5.
- Hassan, E.A., Hassan, E.A. and Hamad, E.H. (2010) Microbial solubilization of phosphate-potassium rocks and their effect on khella (*Ammi visnaga*) growth. *Annual Agricultural Sciences* **55**, 37-53.
- Hazell, P., Wood, S. (2008) Drivers of change in global agriculture. *Philosophical Transactions of the Royal Society B-Biological Science* **363**, 495-515.

- Health Protection Agency (2007) National Standard Method W 1 - General technique for the detection of bacteria by membrane filtration. London: Health Protection Agency.
- Heffer, P., Prudhomme, M. (2010) Fertilizer outlook 2010–2014. In: Heffer P, Prud'homme M, editors. 78th IFA Annual Conference Summary Report; 2010 <http://www.fertilizer.org>. Paris
- Hillel, M. (2008) Balanced crop nutrition: fertilizing for crop and food quality, *Turkish Journal of Agriculture and Forestry* **32**, 183-193.
- Hinsinger, P., Bolland, M.D.A. and Gilkes, R.J. (1996) Silicate rock powder: effect on selected chemical properties of a range of soils from Western Australia and on plant growth as assessed in a glasshouse experiment. *Fertilizer Research* **45**, 69-79.
- Hoflich and Kuhn (1996) Forderung des Wachstums und der Nährstoffaufnahme bei kurziferen Öl- und Zwischenfrüchten durch inokulierte Rhizosphären mikroorganismen. *Zeitschrift für Pflanzenernährung und Bodenkunde* **159**, 575-578.
- Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T. and Williams, S.T. (1994) *Bergey's Manual of Determinative Bacteriology*, 9th edition, Williams and Wilkins, Baltimore.
- Holthusen, D., Peth, S. and Horn, R. (2010) Impact of potassium concentration and matric potential on soil stability derived from rheological parameters. *Soil and Tillage Research* **111**, 75-85.
- Hu, X., Chen, J. and Guo, J. (2006) Two phosphate and potassium solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World Journal Microbiology and Biotechnology* **22**, 983-990.
- Iqbal, M.A., Khalid, M., Shahzad, S.M., Ahmad, M., Soleman, N. and Akhtar, N. (2012) Integrated use of *Rhizobium leguminosarum*, plant growth promoting rhizobacteria and enriched compost for improving growth, nodulation and yield of lentil (*Lens culinaris* Medik.). *Chilean Journal Agricultural Research* **72**, 104-110.
- Jackson, M.L. (1973) *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd, New Delhi, pp. 48-302.

- Jha, P. and Kumar, A. (2009) Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. *Microbial Ecology* **58**, 179-188.
- Kafkafi, U. (1990) The functions of plant K in overcoming environmental stress situations. In: Development of K-fertilizer recommendations: *Proceedings 22nd Colloquium of the International Potash Institute*, pp 81-93.
- Kang, S.M., Khan, A.L., Waqas, M., You, Y.H., Kim, J.H., Kim, J.G., Hamayun, M., and Lee, I.J. (2014) Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. *Journal Plant Interaction* **9**, 673-682.
- Kanwar, J.S. and Chopra, S.L. (1988) Analytical agricultural chemistry. *Kalyani Publisher*, New Delhi.
- Khaliq, A., Abbasi, M.K. and Hussain, T. (2006) Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresource Technology* **97**(8), 967-972.
- Khan, A.G., (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology* **18**, 355-364.
- Khan, M.S., Zaidi, A., Wani, P.A. and Oves, M. (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters* **7**, 1-19.
- Kohler, U. and Luniak, M. (2005) Data inspection using biplots. *The Stata Journal* **5**, 208-223.
- Krauss A. 2003. The International Potash Institute in retrospect and prospect. In: Johnston AE, editor. *Proceedings of the IPI Golden, Jubilee Congress 1952-2003*, 8-10.
- Kulkarni, S. and Nautiyal, C.S. (1999) Characterization of high-temperature tolerant rhizobia isolated from *Prosopis juliflora* grown in alkaline soil. *Journal of Genetics and Applied Environmental Microbiology* **56**, 444-450.
- Kumar, A., Bahadur, I., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Singh, D.K., Dixit, J., (2015) Does a plant growth-promoting rhizobacteria enhance

- agricultural sustainability? *Journal of Pure and Applied Microbiology* **9** (1), 715-724.
- Kumar, A., Maurya, B.R. and Raghuwanshi, R. (2014) Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatalysis and Agricultural Biotechnology* **3**, 121-128.
- Kumar, P., Dubey, R. and Maheshwari, D. (2012) *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research* **167**, 493-499.
- Lack, A., Evans, J. and David, E. (2005) Bios instant notes plant biology. *Taylor & Francis* p. 351.
- Lai, W.A., Rekha, P.D., Arun, A.B. and Young, C.C. (2008) Effect of mineral fertilizer, pig manure, and *Azospirillum rugosum* on growth and nutrient contents of *Lactuca sativa* L. *Biology and Fertility of Soils* **45**, 155-164. DOI 10.1007/s00374-008-0313-3.
- Leaungvutiviroj, C., Ruangphisarn, P., Hansanimitkul, P., Shinkawa, H. and Sasaki, K. (2010) Development of a new biofertilizer with a high capacity for N₂ fixation, phosphate and potassium solubilization and auxin production. *Bioscience, Biotechnology, and Biochemistry* **74**, 1098-1101.
- Leigh, R.A. and Jones, R.G.W. (1984) A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. *New Phytologist* **97**, 1-13.
- Leyval, C. and Berthelin, J. (1989) Interaction between *Laccaria laccata*, *Agrobacterium radiobacter* and beech roots: influence on P, K, Mg and Fe mobilization from minerals and plant growth. *Plant and Soil* **117**, 103-110.
- Li, F.C., Li, S., Yang, Y.Z. and Cheng, L.J. (2006) Advances in the study of weathering products of primary silicate minerals, exemplified by mica and feldspar. *Acta Petrolium Mineral* **25**, 440-448.
- Lian, B., Fu, P.Q., Mo, D.M. and Liu, C.Q. (2002) A comprehensive review of the mechanism of potassium release by silicate bacteria. *Acta Mineral Sinica* **22**, 179-183.

- Lian, B., Wang, B., Pan, M., Liu, C. and Teng, H.H. (2008) Microbial release of potassium from K-bearing minerals by thermophilic fungus *Aspergillus fumigatus*. *Geochimica et Cosmochimica Acta* **72**, 87-98.
- Lin, Q., Rao, Z., Sun, Y., Yao, J. and Xing, L. (2002) Identification and practical application of silicate-dissolving bacteria. *Agricultural Science in China* **1**, 81-85.
- Liu, D., Lian, B. and Dong, H. (2012) Isolation of *Paenibacillus* spp. and assessment of its potential for enhancing mineral weathering. *Geomicrobiology Journal* **29**(5), 413-421.
- Liu, W., Xu, X., Wu, S., Yang, Q., Luo, Y. and Christie, P. (2006) Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. *Environmental Geochemistry and Health* **28**, 133-140.
- Loper, J.E. and Scroth, M.N. (1986) Influence on bacterial sources on indole-3-acetic acid on root elongation of sugarbeet. *Phytopathology* **76**(4), 386-389.
- Maurya, B.R., Kumar, A., Raghuwanshi, R., Bahadur, I. and Meena, V.S. (2015) Effect of phosphate solubilizing isolates on growth, yield and phosphate acquisition by rice and wheat crops. *African Journal of Microbiology Research* **9** Vol. 9(XX).
- Maurya, B.R., Meena, V.S. and Meena, O.P. (2014) Influence of Inceptisol and Alfisol's potassium solubilizing bacteria (KSB) isolates on release of K from waste mica. *Vegetos* **27**, 181-187.
- McLean, E.O. and Watson, M.E. (1985) Soil measurement of plant-available potassium. p 277-278. In: Munson RD (ed): Potassium in agriculture. *American Society of Agronomy Journal Madison, WI*.
- Meena, O.P., Maurya, B.R. and Meena, V.S. (2013) Influence of K-solubilizing bacteria on release of potassium from waste mica. *Agriculture for Sustainable Development* **1**, 53-56.
- Meena, V.S., Maurya, B.R. and Verma, J.P. (2014b) Does a rhizospheric microorganism enhance K⁺ availability in agricultural soils? *Microbiological Research* **169**, 337-347.
- Meena, V.S., Maurya, B.R., Verma, J.P., Aeron, A., Kumar, A., Kim, K. and Bajpai, V.K. (2015) Potassium solubilizing rhizobacteria (KSR): Isolation,

- identification, and K-release dynamics from waste mica. *Ecological Engineering* **81**, 340-347.
- Meena, V.S., Maurya, B.R. and Bahadur, I. (2014a) Potassium solubilization by bacterial strain in waste mica. *Bangladesh Journal of Botany* **43**(2), 235-237.
- Mengel, K. and Kirkby, E.A. (1987) Principles of plant nutrition. 4th Edition: *International Potash Institute*, IPI, Bern, Switzerland. p. 685.
- Mengel, K. and Kirkby, E.A. (2001) Principles of plant nutrition. 5th Edition Dordrecht: Kluwer Acad. Publishers pp. 849.
- Mikhailouskaya, N. and Tcherhysh, A. (2005) K-mobilizing bacteria and their effect on wheat yield. *Latvian Journal of Agronomy* **8**, 154-157.
- Mikled, C., Jiraporncharoen, S. and Potikanond, N. (1994) Fermented slurry as fertilizer for the production of forage crops. Final Report, Thai-German Biogas Application Programme. *Fertilizer Research* **8**, 297-306.
- Miransari, M. and Smith, D.L. (2014) Plant hormones and seed germination. *Environmental and Experimental Botany* **99**, 110-121.
- Mirminachi, F., Zhang, A., Roehr, M. (2002) Citric acid fermentation and heavy metal ions. *Acta Biotechnology* **22**, 363-373.
- Mirza, M.S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P. and Malik, K.A. (2001) Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in-vitro*. *Plant and Soil* **237**, 47-54.
- Mirzaei, A., Vazan, S. and Naseri, R. (2010) Response of yield components of safflower (*Carthamus tinctorius* L.) to seed inoculation with *Azotobacter* and *Azospirillum* and different nitrogen levels under dry land condition. *World Applied Science Journal* **11**(10), 1287-1291.
- Mitter, B., Brader, G., Afzal, M., Compant, S., Naveed, M., Trognitz, F. and Sessitsch, A. (2013) Advances in elucidating beneficial interactions between plants, soil and bacteria. In: Sparks DL, et al., editors; *Advances in Agronomy* **121**, 381-445.

- Mohammadi, K. and Sohrabi, Y. (2012) Bacterial biofertilizers for sustainable crop production: a review. *ARPJ Journal of Agriculture and Biological Science* **7**, 307-316.
- Mora, V., Baigorri, R., Bacaicoa, E., Zamarreño, A.M. and García-Mina, J.M. (2012) The humic acid-induced changes in the root concentration of nitric oxide, IAA and ethylene do not explain the changes in root architecture caused by humic acid in cucumber. *Environmental and Experimental Botany* **76**, 24-32.
- Moritsuka, N., Yanai, J. and Kosaki, T. (2004) Possible processes releasing non-exchangeable potassium from the rhizosphere of maize. *Plant and Soil* **258**, 261-268.
- Muentz, (1890) Surla décomposition des roches et la formation de la terre arable. *CR Academic Science* **110**, 1370-1372.
- Muralikannan, N. (1996) Biodissolution of silicate, phosphate and potassium by silicate solubilizing bacteria in rice ecosystem. M.Sc. (Ag) thesis submitted to *Tamil Nadu Agricultural University, Coimbatore*. p.125.
- Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A. and Ashraf, M. (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances* **32**, 429-448. doi: 10.1016/j.biotechadv.2013.12.005.
- Narde, G., Kapley, A. and Purohit, H.J. (2004) Isolation and characterization of *Citrobacter* strain HPC 255 for broad range substrate specificity for chlorophenol. *Current Microbiology* **48**, 419-423.
- Nieves-Cordones, M., Aleman, F., Martinez, V. and Rubio, F. (2014) K⁺ uptake in plant roots. The systems involved their regulation and parallels in other organisms. *Journal of Plant Physiology* **171**, 688-695.
- Oborn, I., Andrist-Rangel, Y., Askegaard, M., Grant, C.A., Watson, C.A., Edwards, A.C. (2005) Critical aspects of potassium management in agricultural systems. *Soil Use and Management* **21**, 102-112.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L. (1965) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S.D.A. Circ. 939. (U.S. Govt. Printing Office: Washington, DC).

- Omeara, R.A.Q. (1931) A simple delicate and rapid method of detecting the formation of acetyl methyl carbinol by bacteria fermenting carbohydrate. *Journal of Pathology and Bacteriology* **34**, 401-406.
- Paleg, L.G. (1965) Physiological effects of gibberellins. *Annual Review of Plant Physiology* **16**, 291-322.
- Parmar, P. and Sindhu, S. (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. *Journal of Microbiological Research* **3**, 25-31.
- Peix, A., Rivas-Boyer, A.A., Mateos, P.F., Rodríguez-Barrueco, C., Martínez-Molina, E. and Velázquez, E. (2001). Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biology and Biochemistry* **33**, 103-110.
- Phua, C.K.H., Wahid, A.Z. and Rahim, A.K. (2012) Development of multifunctional biofertilizer formulation from indigenous microorganisms and evaluation on their N₂-fixing capabilities on Chinese cabbage using 15N tracer technique. *Pertanika Journal of Tropical Agricultural Science (JTAS)* **35**(3), 667-674.
- Prabha, J. (2006) India's fertiliser imports set to hit the roof on capacity crunch. *The Economic Times*. November, 20.
- Prajapati, K. and Modi, H. (2012) Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. *CIB Technology Journal of Microbiology* **1**, 8-14.
- Prajapati, K., Sharma, M.C. and Modi, H.A. (2012) Isolation of two potassium solubilizing fungi from ceramic industry soils. *Life Science Leaflets* **5**, 71-75.
- Prajapati, K., Sharma, M.C. and Modi, H.A. (2013) Growth promoting effect of potassium solubilizing microorganisms on *Abelmoscus esculantus*. *International Journal of Agricultural Sciences* **3**, 181-188.
- Rajawat, M.V.S., Singh, S., Singh, G. and Saxena, A.K. (2012) Isolation and characterization of K-solubilizing bacteria isolated from different rhizospheric soil. In: Proceeding of 53rd Annual Conference of Association of Microbiologists of India, p. 124.
- Ramamurthy, B., Bajaj, J.C. (1969) Soil fertility map of India. *Indian Agricultural Research Institute, Annual Report* New Delhi.

- Ramarethinam, S. and Chandra, K. (2005) Studies on the effect of potash solubilizing/mobilizing bacteria *Frateruria aurantia* on brinjal growth and yield. *Pestology* **11**, 35-39.
- Rangaswami, G. (1975) Diseases of crop plants in India. Prentice Hall (P) Ltd., New Delhi. p. 520.
- Rengel, Z. and Damon, P.M. (2008) Crops and genotypes differ in efficiency of potassium uptake and use. Special Issue: Special Topics in Potassium and Magnesium Research, *Physiologia Plantarum* **133**(4), 624-636.
- Rich, C.I. (1968) Mineralogy of soil potassium. In: Kilmer VJ, et al., Editors. The role of potassium in agriculture. Madison, WI: ASA, CSSA, SSSA. p. 79-108.
- Romheld, V., Kirkby, E.A. (2010) Research on potassium in agriculture: needs and prospects. *Plant and Soil* **335**, 155-180.
- Rosa-Magri, M.M., Avansini, S.H., Lopes-Assad, M.L., Tauk-Tornisielo, S.M. and Ceccato-Antonini, S.R. (2012) Release of potassium from rock powder by the yeast *Torulaspora globosa*. *Brazilian Archives of Biology and Technology* **55**, 577-582.
- Saeed, I.N, Abbasi, K. and Kazim, M. (2001) Response of maize (*Zea mays*) to nitrogen and phosphorus fertilization under agro-climatic condition of Rawalokot Azad Jammu and Kashmir. *Pakistan Journal Biological Sciences* **4**, 53-55.
- Sangeeth, K.P., Bhai, R.S. and Srinivasan, V. (2012) *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. *Journal of Spice Aromatic Crops* **21**, 118-124.
- Schiavon, M., Pizzeghello, D., Muscolo, A., Vaccaro, S., Francioso, O. and Nardi, S. (2010) High molecular size humic substances enhance phylpropanoid metabolism in maize (*Zea mays* L.). *Journal of Chemical Ecology* **36**, 662-669.
- Seeley, W., and Van Demark, J. (1981) Microbes in action. A laboratory Manual of Microbiology. The W.H. Freeman and Company Inc. (3rd Edition), Sanfrancisco.
- Shaaban, E.A., El-Shamma, I.M.S., El Shazly, S., El-Gazzar, A., Abdel-Hak, R.E. (2012) Efficiency of rock-feldspar combined with silicate dissolving bacteria

- on yield and fruit quality of valencia orange fruits in reclaimed soils. *Journal of Applied Science and Research* **8**, 4504-4510.
- Sheng, X., He, L. and Huang, W. (2001) The conditions of releasing potassium by a silicate dissolving bacterial strain NBT. *Agricultural Sciences in China* **1**(6), 662-666.
- Sheng, X.F. (2002) Study on the conditions of potassium release by strain NBT of silicate bacteria scientia. *Agricultura- Sinica* **35**(6), 673-677.
- Sheng, X.F. (2005) Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. *Soil Biology and Biochemistry* **37**, 1918-1922.
- Sheng, X.F. and He, L.Y. (2006) Solubilization of potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiology* **52**, 66-72.
- Sheng, X.F., He, L.Y. and Huang, W. (2002) The conditions of releasing potassium by a silicate dissolving bacterial strain NBT. *Agricultural Sciences in China* **1**, 662-666.
- Sheng, X.F., Huang, W.Y. (2002) Mechanism of potassium release from feldspar affected by the strain NBT of silicate bacterium. *Acta Pedologica Sinica* **39**(6), 863-871.
- Sheng, X.F., Zhao, F., He, H., Qiu, G. and Chen, L. (2008) Isolation, characterization of silicate mineral solubilizing *Bacillus globisporus* Q12 from the surface of weathered feldspar. *Canadian Journal of Microbiology* **54**, 1064-1068.
- Siddiqui, Z.A. (2006) PGPR: Prospective biocontrol agents of plant pathogens. In: Z.A. Siddiqui (ed). PGPR: Biocontrol and Biocontrol. *Springer, Netherlands*, pp. 112-142.
- Silini-Cherif, H., Silini, A., Ghoul, M. and Yadav, S. (2012) Isolation and characterization of plant growth promoting traits of a rhizobacteria: *Pantoea agglomerans* Ima2. *Pakistan Journal of Biological Sciences* **15**, 267-276.
- Simmons, J.S. (1976) A culture method for differentiating organisms of typhoid color aerogenes group and for isolation of certain fungi. *Journal of Infection and Disaftion* **39**, 209-210.

- Sindhu, S.S., Parmar, P. and Phour, M. (2012) Nutrient cycling: potassium solubilization by microorganisms and improvement of crop growth. In: Geomicrobiology and biogeochemistry: Soil biology. Parmar, N. and Singh, A., Eds. *Springer-Wien/New York*, Germany.
- Sindhu, S.S., Verma, M.K. and Mor, S. (2009) Molecular genetics of phosphate solubilization in rhizosphere bacteria and its role in plant growth promotion. In: Khan MS, Zaidi A (eds) Phosphate solubilizing microbes and crop productivity. *Nova Science Publishers*, USA. pp. 199-228.
- Singh, G., Biswas, D.R. and Marwah, T.S. (2010) Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (*Zea mays*) and wheat (*Triticum aestivum* L.). *Journal of Plant Nutrition* **33**, 1236-1251.
- Singh, R.K., Mishra, R.P.N., Jaiswal, H.K., Kumar, V., Pandey, S.P., Rao, S.B. and Annapurna, K. (2006) Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. *Current Microbiology* **52**, 345-349.
- Sleator, R.D., Hill, C. (2002) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. *FEMS Microbiological Review* **26**, 49-71.
- Smibert, R.M. and Kreig, N.R. (1981) General characterization. In: Methodology for General Bacteriology (Ed.) P. Gerhardt. *Academic Publisher*, New York. pp. 400-450.
- Son, T.T.N., Diep, C.N., Giang, T.T.M. (2006) Effect of *Bradyrhizobia* and phosphate solubilizing bacteria application on soybean in rotational system in the Mekong delta. *Omon Rice* **14**, 48-57.
- Sparks, D.L. (1987) Potassium dynamics in soils. *Advances in Soil Science* **6**, 1-63.
- Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T. and Summer, M.E. (1996) *Methods of Soil Analysis*. Part 3: Chemical Methods, 3rd Edition *Soil Science Society of America and American Society of Agronomy*, Madison, WI, pp. 46-64.
- Srinivasrao, C.H., Satyanarayana, T. and Venkateswarulu, B. (2011) Potassium mining in Indian agriculture: Input and output balance. *Karnataka Journal of Agricultural Science* **24**, 20-28.

- Stevenson, F.J. (2005) Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. *John Wiley and Sons*, New York.
- Stillings, L.L., Drever, J.L., Brantley, S.L., Sun, Y. and Oxburgh, R. (1996) Rates of feldspar dissolution at pH 3–7 with 0–8 mM oxalic acid. *Chemical Geology* **132**, 79-90.
- Styriakova, I., Styriak, I. and Sasvari, T. (2004) Extraction of elements from sulphide and silicate concentrates by selected *Bacillus* isolates. *Metalurgija* **43**, 293-297.
- Subbiah, B.V. and Asija G.L. (1956) A rapid processor of determination of available nitrogen in soil. *Current Science* **25**, 259-260.
- Subhashini, D.V. and Kumar, A.V. (2014) Phosphate solubilizing *Streptomyces* spp. obtained from the rhizosphere of *Ceriops decandra* of Corangi mangroves. *Indian Journal of Agricultural Sciences* **84**(5), 560-564.
- Sugumaran, P. and Janarthnam, B. (2007) Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World Journal of Agricultural Sciences* **3**(3), 350-355.
- Suke, S.N., Deotale, R.D., Priyanka Hiradeve, Mitali Deogirkar and Sorte, S.N. (2011) Effect of nutrients and biofertilizers of chemical and biochemical parameters of maize (*Zea mays* L.). *Journal of Soils and Crops* **21**(1), 107-112.
- Supanjani, Han, H.S., Jung, S.J. and Lee, K.D. (2006) Rock phosphate potassium and rock solubilizing bacteria as alternative sustainable fertilizers. *Agronomy for Sustainable Development* **26**, 233-240.
- Torres, A.R., Araújo, W.L., Cursino, L. Hungria, M., Plotegher, F., Mostasso, F.L. and Azevedo, J.L. (2008) Diversity of endophytic *Enterobacteria* associated with different host plants. *Journal of Microbiology* **46**, 373-379.
- Troufflard, S., Mullen, W., Larson, T.R., Graham, I.A., Crozier, A., Amtmann, A. and Armengaud, P. (2010) Potassium deficiency induced the biosynthesis of oxylipins and glucosinolates in *Arabidopsis thaliana*. *BMC Plant Biology* **10**, 172-184. doi:10.1186/1471-2229-10-172.

- Uroz, S., Calvaruso, C., Turpault, M.P. and Frey-Klett, P. (2009) Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology* **17**, 378-387.
- Uroz, S., Calvaruso, C., Turpault, M.P., Pierrat, J.C., Mustin, C. and Frey-Klett, P. (2007) Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied and Environmental Microbiology* **73**, 3019-3027.
- Vahedi, A. (2013) The investigation of arbuscular mycorrhizal fungal effect on growth and nutrients in *Trifolium pratense* in the multi metals contaminated soil. *International Journal of Biology* **5**, 71-80.
- Vandevivere, P., Welch, S.A., Ullman, W.J. and Kirchman, D.J. (1994) Enhanced dissolution of silicate minerals by bacteria at near neutral pH. *Microbiology and Ecology* **27**, 241-251.
- Veresoglou, S.D., Mamolos, A.P., Thornton, B., Voulgari, O.K., Sen, R. and Veresoglou, S. (2011) Medium-term fertilization of grassland plant communities masks plant species-linked effects on soil microbial community structure. *Plant and Soil* **344**, 187-196.
- Vessey, K.J. (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **25**, 557-586.
- Walkley, A., Black, I.A. (1934) Rapid titration method for organic carbon of soils. *Soil Science* **37**, 29-32.
- Wang, J.G., Zhang, F.S., Cao, Y.P., Zhang, X.L. (2000) Effect of plant types on release of mineral potassium from gneiss. *Nutrient Cycle and Agroecosystem* **56**, 37-44.
- Welch, S.A. and Ullman, W.J. (1993) The effect of organic acids on plagioclase dissolution rates and stoichiometry. *Geochimica et Cosmochimica Acta* **57**, 2725-2736.
- White, P.J. and Karley, A.J. (2010) Potassium. In: Hell R, Mendel RR (eds) Cell biology of metals and nutrients, plant cell monographs, *Berlin: Springer* **17**, 199-224.

- Wood, C.W., Reeves, D.W. and Himelrick, D.G. (1993) Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status and crop yield: A review. *Proceedings of Agronomy Society New Zealand* **23**, 1-9.
- World Bank. (2008) World development report on agriculture for development. Washington, DC: The World Bank; 2007.
- Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. and Wong, M.H. (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma* **125**, 155-166.
- Xie, J.C. (1998) Present situation and prospects for the world's fertilizer use. *Plant Nutrition and Fertilizer Science* **4**, 321-330.
- Xiufang, H., Jishuang, C. and Jiangfeng, G. (2006) Two phosphate and potassium solubilizing bacteria isolated from Tianmu Mountain Zhejiang, China. *World Journal of Microbiology and Biotechnology* **22**, 983-990.
- Yadegari, M., Farahani, G.H.N. and Mosadeghzad, Z. (2012) Biofertilizers effects on quantitative and qualitative yield of Thyme (*Thymus vulgaris*). *African Journal of Agricultural Research* **7**, 4716-4723.
- Yakhontova, K., Andreev, P.I., Ivanova, M.Y. and Nesterovich, L.G. (1987) Bacterial decomposition of smectite minerals. *Doklady Akademii Nauk, SSR* **296**, 203-206.
- Yousefi, A.A., Khavazi, K., Moezi, A.A., Rejali, F. and Nadian, N.H. (2011) Phosphate solubilizing bacteria and arbuscular mycorrhizal fungi impacts on inorganic phosphorus fractions and wheat growth. *World Applied Science Journal* **15**(9), 1310-1318.
- Youssef, G.H., Seddik, W.M.A. and Osman, M.A. (2010) Efficiency of natural minerals in presence of different nitrogen forms and potassium dissolving bacteria on peanut and sesame yields. *American Journal of Science* **6**, 647-660.
- Zahra, M.K., Monib, M.S., Abdel-Al, I. and Heggo, A. (1984) Significance of soil inoculation with silicate bacteria. *Zentralbl Mikrobiology* **139**(5), 349-357.
- Zakaria, A.A.B. (2009) Growth optimization of potassium solubilizing bacteria isolated from biofertilizer. Malaysia Pahang: Bachelor of Chemical

- Engineering (Biotechnology), *Faculty of Chemical, Natural Resources Engineering University* pp. 40.
- Zandonadi, D.B., Santos, M.P., Dobbss, L.B., Olivares, F.L., Canellas, L.P., Binzel, M.L., Okorokova-Façanha, A.L. and Façanha, A.R. (2010) Nitric oxide mediates humic acids-induced root development and plasma membrane H⁺-ATPase activation. *Planta* **231**, 1025-1036.
- Zarjani, J.K., Aliasgharzad, N., Oustan. S., Emadi, M. and Ahmadi, A. (2013) Isolation and characterization of potassium-solubilizing bacteria in some Iranian soils. *Archives of Agronomy and Soil Science* **59**, 1713-1723.
- Zeng, X., Liu, X., Tang, J., Hu, S., Jiang, P. and Li, W. (2012) Characterization and potassium-solubilizing ability of *Bacillus circulans* Z1-3. *Advances of Science Letters* **10**, 173-176.
- Zhang, A., Zhao, G., Gao, T., Wang, W., Li, J. and Zhang, S. (2013) Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: a soil microorganism with biological control potential. *African Journal of Microbiological Research* **7**(1), 41-47.
- Zhang, C., Kong, F. (2014) Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. *Applied Soil Ecology* **82**, 18-25.
- Zhao, F., Sheng, X., Huang, Z. and He, L. (2008) Isolation of mineral potassium-solubilizing bacterial strains from agricultural soils in Shandong Province. *Biodiversity Science* **16**, 593-600.
- Zhou, H., Zeng, X., Liu, F., Qiu, G. and Hu, Y. (2006) Screening, identification and desilication of a silicate bacterium. *Journal of Central South University Technology* **13**, 337-341.
- Zuniga, A., Poupin M. J., Donoso R., Ledger T., Guiliani N., Gutierrez R. A. and González, B. (2013) Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of *Arabidopsis thaliana* by *Burkholderia phytofirmans* PsJN. *Molecular Plant-Microbe Interactions* **26**(5), 546-553. doi.org/10.1094/MPMI-10-12-0241-R.

Websites Consulted:

www.fao.org/home/en

www.iari.res.in

www.icar.org.in

www.ikisan.com

www.incitecpivot.com.au

www.infomine.com

www.ipni.net

www.isss-india.org

www.ncbi.nlm.nih.gov

www.sobip.com
